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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-NINTH ANNUAL MEETING OF THE SOCIETY, CHICAGO, ILLINOIS DECEMBER 28 TO 30, 1947

The relation of pH to sporulation and growth of Gibberella zeae on agar and in liquid media. ANDERSEN, A. L. Sporulation and growth of *Gibberella zeae* are directly influenced by the initial pH of the culture medium, whether solid or liquid. The composition of the medium, particularly with respect to nutrient concentration, and nitrogen source, is also important, in part because of indirect effects on the pH changes that occur as growth progresses. On KNO_3-agar media, maximum sporulation resulted if the initial pH was approximately 3.0–3.3, whereas on NH_4NO_3-agar media an initial pH of 4.3 was optimum. In similar liquid media, maximum sporulation occurred in those media initially adjusted to pH 5–6. Nearly all the media shifted in pH as growth progressed. On KNO_3-agar media, this period of pH change was closely correlated with the period of rapid spore development; with those media having an initial pH below 7, the pH shift was toward alkalinity; those having an initial pH above 7 shifted first toward neutrality and then back toward the initial value. Media high in carbon and nitrogen produced more mycelium and fewer spores than did similar media low in these ingredients. Moreover, the optimum initial pH for mycelium production was higher for the low carbon and nitrogen media than in the case of media high in these two elements.

An evaluation of certain substituted phenyl esters for the treatment of cotton seed. ARNDT, C. H. Cotton seed naturally infested by *Colletotrichum gossypii* was treated with various substituted phenyl esters and the seed germinated in sand culture. At comparable dosages, the acetate ester of 2,4,5-trichlorophenol was effective in preventing seedling infection by *C. gossypii;* the formate and propionate were slightly less effective; while the carbonate, succinate, and laurate were ineffective. The substitution of bromine for chlorine caused little change in effectiveness. The nature of the chlorination of the phenyl acetate also influenced the fungicidal properties. The 2,3,4,6-tetrachlorophenyl acetate was more toxic to both the fungus and the host plant than the 2,4,5-trichlorophenyl acetate; while the chemicals with the Cl atoms in the 2,4,6, the 2,3,6, and the 2,3,4,5,6 positions were somewhat less effective. The latter was the most toxic of these chemicals to the host plant. The ortho, para, and the 2,4-dinitrophenyl acetates were not fully effective fungicides. Results obtained in field plantings, which were generally in agreement with the laboratory results, indicated that 2,4,5-trichlorophenyl acetate at a dosage of 1 gm./kg. of seed may be a satisfactory treatment for fuzzy cotton seed.

Inheritance of resistance to spot blotch of barley. ARNY, DEANE C. In seedling tests of F_2 and F_3 generations of several crosses, the difference between susceptibility and resistance to spot blotch (*Helminthosporium sativum*) appeared to be due to a single-factor pair, with susceptibility dominant. There was no detectable association of spot-blotch reaction with lemma and pericarp color (black vs. white), awn barbing or rachilla hair length, or reaction to stem rust. An association between spot-blotch resistance and the xantha character of Colsess IV was suggested in limited tests. In one cross spot-blotch susceptibility appeared to be associated with stripe (*Helminthosporium gramineum*) resistance.

Molds on apple storage containers and their control. ATKINS, J. G., JR. The growth of various molds on apple containers kept at high humidities is one of the problems associated with storage. *Penicillium expansum, Cladosporium herbarum, Stemphylium consortiale, Mucor plumbeus,* and *Cephalosporium* sp. were the most prevalent on new containers after several months in five western New York storage houses. Several other fungi were also encountered. *Mucor racemosus* was most frequently isolated from containers used in previous seasons on which the ''whiskers'' type of mold predominated. *Mucor plumbeus, Rhizopus nigricans,* and *Mucor* sp. were often present. Of the fungi commonly isolated from moldy containers only *Penicillium expansum* produced a rapid rot of Cortland apples. Other rot-producing organisms included *Botrytis cinerea, Aspergillus niger, Rhizopus nigricans,* and *Mucor* sp. A large number of chemicals were evaluated as mold inhibitors in laboratory tests. In such tests silver and mercury compounds were best followed by the Dowicides, Cuprinol, Thiosan, Copper 8-quinolinolate, and Spergonex. In storage tests Dowicides B, F, G and S, Thiosan, Spergonex, Cuprinol, Roccal, Phygon, and silver nitrate gave good control. Apples in direct contact with treated containers were injured by Spergonex, Cuprinol, and Dowicides B, F, G and S.

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Physiological specialization in Cladosporium fulvum. BAILEY, D. L. The parasitic capabilities of the 8 physiologic races of *Cladosporium fulvum*, which have been reported to date in Ontario, Canada, are indicated in terms of reactions of the following differential host varieties: Red Currant (*Lycopersicon pimpinellifolium*); Vetomold; V–121; Stirling Castle; V–473; *L. hirsutum*; *L. hirsutum* var. *glabratum*. Evidence is offered that the evolution in virulence encountered in *C. fulvum* has been a function of the increasing resistance of the host varieties to which it has been exposed.

Time and locus of infection of American elms in relation to extent of vascular invasion by Ceratostomella ulmi. BANFIELD, W. M. Separate lots of American elm seedlings 18 to 28 feet in height were inoculated at intervals of one month, from April to September. Two holes per tree were cut to the cambium with a ¼-inch leather punch. The holes were packed with sawdust media containing *Ceratostomella ulmi* and then were taped. Less than 3 per cent of 318 inoculations made April 25 resulted in general vascular invasion. Fifty-three per cent of 146 inoculated May 10, 91 per cent of 103 inoculated June 7, and 70 per cent of 80 inoculated July 26 developed general vascular invasion and wilt. None of the 90 trees inoculated September 5 developed general vascular invasion. Similar inoculations made in apical shoots, 1- or 2-year stems, at comparable periods yielded various results. Inoculations made at any time in new shoots resulted in only limited invasion with inconspicuous wilt. Inoculations made in 1- or 2-year stems prior to May 10 or after September 1 resulted in only limited invasion in 850 trials. Five per cent of 199 inoculations made in June in 1-year stems and 17 per cent of 185 made in 2-year stems resulted in general invasion. Such invasion occurred in only 2 per cent of 167 inoculations made in July on 2-year stems and in none of 197 made then in 1-year stems.

Induced oospore production in the genus Phytophthora. BARRETT, J. T. It is well known that oospore production in certain species of *Phytophthora* is rare or erratic, or entirely unknown. In other species certain isolates give positive results, others negative. Pairing of different isolates of the same species and of different species by several students of the genus have resulted in stimulating oospore production. By the use of two isolates of *P. drechsleri*, one determined as male, the other female, and neither producing oospores alone, maleness and femaleness has been established in species which rarely or never produce oospores such as *P. citrophthora*, *P. cinnamomi*, and *P. infestans*. All isolates of *P. citrophthora* and *P. infestans* tested react as males, while those of *P. cinnamomi* react as females. All isolates of *P. cryptogea* from California react as males and one presumably from South Africa reacts as female. No oospores have developed as yet between *P. citrophthora*, female, and *P. cinnamomi* male. These results support, and extend in part, observations reported by Clinton, Leonian, Narasimhan, and Uppal.

Supplementary notations relative to spot anthracnoses (Elsinoë and Sphaceloma) in Europe since 1936. BITANCOURT, A. A., AND ANNA E. JENKINS. Examination of a culture and a leaf specimen discloses the presence in France of an hitherto unrecorded *Sphaceloma* on plum. We consider this most likely to be the same species as that found on plum in the Crimea in 1916 by Garbowski, who later reported it under the name of the related *Hadrotrichum populi* Sacc. Snowberry anthracnose, previously known only from North America, is here reported from France and Denmark. A revision of our 1936 tabulation of spot anthracnoses is offered with respect to present knowledge of their incidence in Europe.

Transmission of clover club-leaf virus through the egg of its insect vector. BLACK, L. M. In experiments started at the Rockefeller Institute, leafhoppers (*Agalliopsis novella*) carrying clover club-leaf virus (*Aureogenus clavifolium*) were allowed to deposit eggs in Grimm alfalfa plants (*Medicago sativa*). Later each of 30 nymphs was removed from the alfalfa as soon as it had hatched so that it had no opportunity to feed on the plant in which the egg was laid. Each nymph was placed on a crimson clover seedling (*Trifolium incarnatum*) and transferred at weekly intervals to a fresh seedling until it died. Three insects died within two weeks. The remaining twenty-seven lived from 13 to 33 weeks. The clover test plants and an equal number of control plants were grown in a greenhouse for at least six weeks after the removal of the insects. Twenty-four of the 27 insects or 89 per cent transmitted the disease. None of the insects infected plants until at least 3 weeks had elapsed from the time of hatching. Best transmission occurred during the 7th to 11th weeks inclusive. Altogether 68 of the 642 test plants and none of the control plants were infected. This is the second instance of a plant virus being transmitted through the egg of its insect vector.

The occurrence and transmission of Little Cherry in Washington. BLODGETT, E. C., E. L. REEVES, C. M. WRIGHT, AND H. E. WILLIAMS. The Little Cherry Disease recognized

in Washington first in 1946 in four orchards in three sections of the State was found to be widely scattered according to the 1947 survey. A total of 172,078 bearing cherry trees (about one-third of the State's total) was examined on 2,458 properties in 16 counties east, and in 5 west, of the Cascade Mts. A total of 289 infected properties was located in 13 counties east of the mountains, which represents a total of 1,471 trees affected or less than 1 per cent of those examined. It is believed that the disease has been in the State for about 5 years. Bud inoculation tests on June 27, August 19, and September 18, 1946 on six healthy Bing and two Lambert trees showed by mid-summer of 1947 that the virus had been definitely transmitted to four Bing and to the two Lambert trees. Of four root inoculated Bing trees, only one had the disease. Control of the disease is being attempted with a voluntary tree removal program.

Leaf rust of wheat in Mexico. BORLAUG, N. E., AND J. RUPERT. Leaf rust of wheat, caused by *Puccinia rubigovera tritici*, has become an increasingly important factor in wheat production in Mexico. Many varieties introduced into Mexico because of their known stem rust resistance have been found exceedingly susceptible to leaf rust under Mexican conditions. Furthermore, there is in Mexico at least one physiologic race of leaf rust not commonly found elsewhere and highly pathogenic to a wide range of varieties, including the durums. Mida and Trigo Supremo × 41–116 have been selected as the best parental material for leaf rust resistance. Newthatch and Kenya, commonly used as parents when stem rust resistance is sought, are highly susceptible to leaf rust races in Mexico.

Further studies on bacterial necrosis of the giant cactus. BOYLE, ALICE M. This paper summarizes the advances made in the study of bacterial necrosis of the giant cactus (*Carnegiea gigantea* (*Cereus giganteus*)) since the initial report before this Society at Philadelphia, 1940, and the publication of a paper on the disease in PHYTOPATHOLOGY in 1942. The disease is distributed over practically the entire habitat of the giant cactus; rapid spread is attributed to the activity of an insect vector now identified and proved culpable; longevity of the pathogen, age of the host with relation to susceptibility, and the astonishing attempts of the host to ''cork-off'' the bacterial lesions have been investigated. Serological studies support cultural, morphological, and other evidence that *Erwinia carnegieana* is an authentic species of bacterium.

Studies on plant teratomas. BRAUN, ARMIN C. When certain moderately virulent strains of the crown-gall bacterium were inoculated into *Kalanchoë* plants at different points along the stem, typical undifferentiated tumors, similar to those found on many other hosts, were produced. As the tumors enlarged those that originated in the younger, less differentiated portions of the stems produced leaves and stems that showed varying degrees of morphological differentiation. Many of these structures were highly distorted; others appeared to be more or less normal. Studies designed to determine whether these structural elements arose from normal host cells or from tumor cells strongly suggested that they were the result of the growth of tumor cells that had recovered in varying degrees from the effects of the tumor-inducing stimulus. The results can perhaps be interpreted by assuming the presence in plant cells of factors that determine the morphogenetic fate of the cells. In those instances, in which undifferentiated tumors developed, the factors concerned with morphogenetic determination must have been completely overwhelmed by the action of the tumor-inducing principle. The relative competence of the cells acted upon may therefore be of fundamental importance in determining the type of structure that ultimately develops.

Cytological effects of penicillin and streptomycin on crown gall. BROWN, J. G. Living cells in crown galls, as well as the concerned bacterial incitor (*Agrobacterium tumefaciens*), are killed by applications of penicillin and streptomycin to the galls; normal cells in and near the resulting necrotic galls remain visibly uninjured. Cytologic study reveals progressive stages in the destruction of the nuclei of the gall cells, including marked enlargement, sometimes to the degree of bursting of the nuclear membrane, followed by shrinkage and deformity; the nucleoplasmic network usually persists during the enlargement and into the stages of shrinking. That twigs, green leaves, and roots on aerial crown galls originate from normal cells, as maintained by Erwin F. Smith, rather than from totipotent gall cells, appears to be strikingly demonstrated in galls treated with the mentioned antibiotics: such organs remain alive for varying periods in galls otherwise necrotic from application of the antibiotics.

Anthracnose of cereals and grasses. BRUEHL, G. W. Anthracnose of Sudan grass and rye affects the major plant structures. In seedlings from infected seed the first internode is invaded, resulting in blighting or later crown infection and rot. Crown-

and culm-rot development diminished during the active vegetative growth of the plants and became active again as the plants matured. Yields were reduced by the crown-culm invasion. Spore production was usually heavy on the blighted plant parts of Sudan grass and less abundant on rye. Temperature response of isolates of *Colletotrichum graminicolum* indicated two groups; the one from *Sorghum* spp. more tolerant to higher temperatures than the cereal and grass isolates. Specialization on host groups was evident. Seedling blight of Sudan grass showed little temperature response between 16° and 32° C. Rye seedling blight and crown damage was reduced below 10° C. Preliminary evidence indicated an important antagonistic effect of soil microflora on *C. graminicolum*.

Aphanomyces cochlioides infestation in irrigated sugar beet–alfalfa rotation plots at Newell, South Dakota. BUCHHOLTZ, W. F. The sugar beet root-infecting fungus, *Aphanomyces cochlioides*, was abundant in plots devoted to a four-year rotation of sugar beets–oats and alfalfa–alfalfa–alfalfa. Plots in two similar four-year rotations, with potatoes or wheat in place of sugar beets, were apparently free of *A. cochlioides*. The continuous beet plot, without added manure or fertilizer, was infested, but not so heavily as those in the four-year alfalfa–sugar beet rotation. Two continuous beet plots, one with phosphate added and one with manure added annually, were apparently free of the organism. Plots planted continuously to alfalfa, potatoes, wheat, barley, oats, or corn were apparently free of *A. cochlioides*. Nonfertilized plots, planted to beets alternating with potatoes or oats, were infested. Plots in three-year sugar beet rotations without legumes were apparently free of the organism. In a six-year rotation of sugar beets–oats and alfalfa–alfalfa–alfalfa–alfalfa–potatoes, the third crop year alfalfa plot was heavily infested with *A. cochlioides*. A crop failure on this plot was successfully predicted in advance of field planting. Soil infestation by *A. cochlioides* apparently was "initiated" by crops of sugar beets, but very much favored by an abundance of alfalfa, a non-host crop, in the rotation.

Strawberry dud. CAMPBELL, LEO. The characteristic symptom of strawberry dud is a dearth of new root development, ranging from almost a full complement of new roots to none at all. Correlated with the root symptoms, an increased red discoloration and reduction in vigor of the tops to complete wilting and death of the plants follow transplanting. Poor stands and weak plants result. The tops of affected plants gradually acquire an open appearance and the younger, central leaves develop lighter green and are shorter than normal. Dud occurs most commonly on light, badly leached soils, innately deficient in nutrients. Faulty methods of handling plants, such as late digging, delayed planting, and poor or prolonged storage aggravate dud. Since the malady occurs where barnyard manure, green manure, or ordinary commercial fertilizers are used in abundance, some minor element deficiency is suspected as a causative factor. Such factors as overheating in the pack will result in symptoms of dud. Growing planting stock on good fertile soil and proper handling of plants after digging are being recommended as control measures, pending the determination of the primary cause of dud.

Effect of temperature on brown stem rot of soybeans. CHAMBERLAIN, D. W., AND W. B. ALLINGTON. The effect of temperature on the development of the brown stem rot of soybeans and on the causal organism was studied in the greenhouse and in the field. Air temperature appeared to be the important factor in disease development. When artificially inoculated plants were maintained at 15°, 21°, and 27° C., internal stem-browning appeared within three weeks in plants at 15° C., while plants at the higher temperatures showed little or no evidence of the disease. When these latter plants were transferred to the 15° C. chamber, they developed symptoms within 2 to 3 weeks. These results are correlated with outdoor temperatures in 1945, 1946, and 1947 and the development of brown stem rot in these years. In culture, the optimum temperature for growth of the fungus was 22–24° C.; for germination of conidia, 21–25° C.; for spore production, 15–20° C.

Relation between spore load on the seed and amount of smut infection in cereal grains. CHEREWICK, W. J. A method of determining smut spore load on cereal seed grain was developed in response to demands of farmers, and also with a view to conserve, during the war years, the short supply of seed disinfectants. This method was soon adopted by commercial firms that examine large numbers of farmers' seed samples and recommend whether or not seed treatment is needed. Greenhouse and field tests indicated that seed bearing spore loads of 1:1,000,000 or less by weight did not require seed treatment for smut control, and these results were used as the basis for the recommendations. Recent experiments revealed, however, that in the case of wheat seed threshed by a machine with rubberized cylinder and concaves, smut balls are frequently not crushed and, therefore, spores are not evenly distributed through the grain. While a sample of

such seed may show only a trace of spores it may develop up to 1 per cent smutted plants, when planted, owing to the presence of occasional smut balls. Moreover, in barley, false loose smut (*Ustilago nigra*) may be carried in part as mycelium under the hull. It would seem, therefore, that cereal seed carrying only a trace of smut spores must be treated for smut control.

Two important diseases of alfalfa in Manitoba. CHEREWICK, W. J. The most prevalent disease in Manitoba affecting both alfalfa and sweet clover is a crown rot which results in heavy losses, particularly in the alfalfa crop. Extensive surveys, isolation and inoculation tests during the past several years indicate that, under conditions prevailing in Manitoba, a strain of *Rhizoctonia solani* is the main, if not the sole, causal agent of the crown rot. The infected plants are readily invaded by saprophytic or weakly parasitic soil fungi which accelerate the disintegration of crown tissues and thus predispose the plants to winter-killing. The dead plants occur in patches which are soon overgrown with weeds and, therefore, damage occurs both from the reduction in forage yield and the infestation of the field with weeds. Another important disease is the "blossom drop" of alfalfa. It is prevalent in alfalfa seed growing districts in Manitoba and other provinces of Canada and frequently results in a complete failure of seed-set. The results of isolation and inoculation tests indicate that this disease may be caused by a species of *Alternaria*. The infected florets wither and fall off regardless of whether they were tripped or not. The addition to the soil of various essential food elements does not seem to have any effect on the disease.

The pathogenicity of Mycosphaerella citrullina. CHIU, W. F. Pycnospores, ascospores, and mycelium of *M. citrullina* were found equally infective on cotyledons and young foliage leaves of muskmelon and Hawkesbury and Improved Kleckley Sweet watermelons. Foliage leaves of cucumber, Hubbard squash, and Table Queen squash were very resistant. Infection of the hypocotyl and the stem of all cucurbits tested occurred only through punctures, through weakened areas, or by extension of lesions on the cotyledon. Cucumber and Hubbard squash were very resistant to formation of cankers on the hypocotyl when young, but both became very susceptible when old. The contrary was true for Table Queen squash. Cankers developed on the stems and hypocotyls of watermelon and muskmelon throughout their growing periods provided moist conditions prevailed. Ten isolates, secured by single-spore isolations and by irradiation, showed a gradation of pathogenicity from very high to none. The optimum temperature for infection of watermelon was 24° C., for muskmelon 16° to 20°. Field experiments revealed that Improved Kleckley Sweet watermelon was decidedly more resistant than Hawkesbury, while Cubit variety of cucumber was highly resistant compared with Stay Green, Chicago Pickling, Parker, Marketer, Long Stay Green, and National Pickling.

The effect of repeated passage of Helminthosporium sativum through the host on genetic variation and pathogenicity. CHRISTENSEN, J. J., AND C. L. SCHNEIDER. A line of *Helminthosporium sativum* derived from many successive monosporous isolations and grown on artificial nutrients for 28 years was passed through Marquis wheat ten successive generations. Disease-free seedlings were grown in test tubes containing water agar and were inoculated with single conidia of the pathogen. Monosporous cultures were made by atomizing the surface of potato-dextrose agar in Petri plates with a dilute suspension of spores from diseased plants after each generation in the host. These cultures were compared with isolates grown continuously on potato-dextrose agar. With the exception of five mutants, 14,400 monosporous progenies from the host were identical in cultural characters with the original line. The frequency of mutation of *H. sativum* on the host was approximately 1:2900. Cultures of the five mutants derived from the host were strikingly different from each other and their parent in color, type and rate of growth, and in amount of sporulation. Pathogenicity tests were made with monosporous isolates of the original line, obtained after five generations on the host, by sowing the seed of wheat and barley in infested soil. The virulence of the line was not changed in five successive passages through wheat plants.

Breeding tobacco for wildfire resistance. CLAYTON, E. E. Resistance to tobacco wildfire was obtained from *Nicotiana longiflora*, a small wild species. A fertile tobacco-like genotype, TL 106, was secured from the first backcross (Nt × Nl × Nt). TL 106 was crossed with various susceptible commercial tobacco varieties and F₂ seed grown. F₁ plants were also backcrossed to the same susceptible varieties. The F₂ populations gave 1,219 susceptible and 232 resistant plants, a ratio of 5.25 to 1. The first backcross populations gave 1,490 susceptible and 36 resistant, a ratio of 41.4 to 1. Tests with more advanced backcrosses showed that there was much variation in the number of resistant plants recovered from populations with identical parentage. The results indicate that

considerable chromosome irregularity still persists as the consequence of the interspecies cross, but suggest that as this is eliminated segregation on a simple basis is likely. The resistance was maintained even in the presence of prolonged, storm induced, leaf water-soaking. Field studies of resistant progeny from third and fourth backcrosses to susceptible tobacco showed that resistance was not linked with any undesirable growth characters.

The use of potato starch in virus purification. COCHRAN, G. W. Active tobacco-mosaic virus was purified by several procedures involving passage through starch columns. The virus was concentrated at the top of the columns by a "salting out" with ammonium sulphate or by a precipitation at the isoelectric point. In either case the passage of a pH 7, 0.1 M phosphate buffer through the starch moved the virus downward so that it could be collected at the bottom of the column as a purified concentrate. A more rapid method using extremely short starch columns was devised to fractionate the ammonium sulphate precipitable constituents of diseased plant juices. The ammonium sulphate concentration, just below the threshold of virus precipitation, was determined. The virus-protein mixture was adjusted to this concentration and passed through a thin starch filter. The salt concentration was then raised to a point slightly above the threshold of precipitation and the virus-protein mixture was passed through a second thin starch filter. The virus was held on the top of this second filter and was washed down with un-salted buffer and obtained as a purified concentrate.

Development of fungicidal aerosols as foliage protectants. COHEN, SYLVAN I. Thirty-four chemicals representing thirteen organic classes were tested for solubility in or compatibility with freon-12, then screened as an aerosol solution for phytotoxicity and fungitoxicity. Promising fungicides were formulated as treatments for the control of five foliage diseases. One aerosol treatment using an organic copper formula produced 96.5–100 per cent protection for 15–30 days against rose black spot, bean powdery mildew, bean anthracnose, and tomato early blight. It also succeeded in eradicating the pathogen from 24-hour-old lesions of bean powdery mildew. Two treatments at 15- and 22-day intervals reduced rust 39.1 to 60.6 per cent on three carnation varieties. A method was found to increase the deposit of aerosol droplets upon under and vertical surfaces sufficient to inhibit the spore germination of two fungi. Certain aerosol deposits exhibited good tenacity, as evidenced by no appreciable loss in fungitoxicity in laboratory tests after 4 minutes of washing. Aging of promising aerosol deposits for 17–30 days produced little or no effect upon their fungitoxic properties. The efficiency of aerosol deposits is indicated by the observation that when only 0.23 per cent of the total surface area was occupied by aerosol droplets of one formula, 100 per cent inhibition of *Stemphylium sarcinaeforme* spore germination resulted.

Forecasting late blight in eastern Virginia in 1947. COOK, HAROLD T. The method of forecasting late-blight epiphytotics described last year was put to a practical test during the 1947 season. Beginning on May 8, the cumulative rainfall and mean temperatures were plotted each day. Forecasts based on the graphs were issued at weekly intervals from May 15 to June 5. All of these forecasts stated that the weather conditions were unfavorable for late blight and that spraying or dusting to control the disease was not necessary at that time. The forecasts proved to be correct. There was no epiphytotic in Eastern Virginia and over 90 per cent of the potato and tomato acreage was entirely free of the disease. The crops also were free of other serious leaf and fruit diseases. Since it would have cost approximately $2,000,000 for routine spraying or dusting of the 60,000 acres of potatoes and tomatoes, the elimination of the needless use of fungicides is of considerable economic importance. A graph showing the moving 7-day average rainfall and temperature was developed to supplement the cumulative graph. The moving graph was more accurate for analyzing the late blight—weather relations over the entire season and also aided in correcting for unusually heavy rainfall early in the forecasting period.

Antibiosis and sugarcane root rot. COOPER, W. E., AND S. J. P. CHILTON. During the past two years, more than 6500 isolates of *Actinomyces* were obtained from Louisiana sugarcane soils. These were tested in culture to determine which ones were antibiotic to *Pythium arrhenomanes,* one of the important fungi causing root rot of sugarcane. The four major soils showed a consistent difference in the number of antibiotic *Actinomyces.* The Yahola silt loams had the highest number, the Yazoo silt loams next, the Iberia silt loams and first terrace soils were third, and the Sharkey clays the lowest. Comparative yield tests during 5 years with sugarcane on the various soils gave yields correlating with the number of antibiotic *Actinomyces.* In greenhouse tests in sterile soil, certain of the *Actinomyces* increased in the soil and reduced root rot in corn. Other strains failed to increase in the soil.

Stem anthracnose of Lima bean and its control. Cox, ROBERT S. Stem anthracnose (*Colletotrichum truncatum*) causes serious losses of Lima beans in North Carolina. Although the most striking symptoms are brick-red lesions on pods, stems, and leaves, lesions commonly occur on the inflorescence, frequently resulting in blossom-drop and death of immature pods. The spore mass is typically gray, but a pink one is occasionally produced. In culture this pinkness is retained indefinitely. Experimental evidence indicates that the fungus is seed-transmitted. Of 16 varieties tested, all were highly susceptible, except Jackson Wonder, which showed some tolerance. Fungicidal sprays and dusts were applied at weekly intervals on replicated plots of the Henderson Bush variety. Phygon spray gave most effective control on foliage, stems and pods. In the spray test, average yields of marketable pods (lbs. per acre) from Phygon (1.5–100), Fermate (3–100), and control plots were 5847, 4803, and 1711, respectively. Of the dusts tested (Phygon, 5 per cent; Dithane Z–78, 6 per cent; Fermate, 10 per cent; Zerlate, 10 per cent; and tribasic copper sulphate, 5 per cent metallic copper), only Phygon and Dithane significantly controlled the disease. Phygon (dust or spray) caused slight leaf chlorosis and blossom-drop. Tribasic copper caused moderate foliage injury.

The effect of temperature and nitrogen source on the development of stem rust of wheat. DALY, J. M. The effect in sand culture of 40 three-salt nutrient solutions on the reactions of three spring wheats to race 56 of *Puccinia graminis tritici* was studied at temperatures above 80° F. At these temperatures, mineral nutrition did not alter stem rust reaction, although nitrogen concentration ranged from 20 to 305 p.p.m. Mindum was resistant (infection types 0; to 1); Marquis was susceptible (infection types 3 to 4) and Thatcher was susceptible (infection types 3 to 4) except in one experiment during a period of low light intensity when Thatcher was resistant (infection types 0; to 1). At temperatures below 70° F., certain forms of nitrogen affected the stem rust reaction of Thatcher, but not Mindum, wheat. Thatcher was mesothetic (infection type X) when grown in a mixture of three parts sand and two parts loam and supplied with 400 p.p.m. of nitrogen as $Ca(NO_3)_2$ or KNO_3; but plants supplied equivalent nitrogen as $(NH_4)_2SO_4$ were resistant (infection types 0; to 1). Applications of NH_4NO_3 resulted in resistance or moderate resistance to stem rust. The pH of the nutrient solution did not change the effect of nitrogen. Applications of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, NH_4NO_3, or urea did not influence either the infection type or percentage of stem rust in field plots.

Inhibition of tobacco streak virus by juice from healthy plants. DIACHUN, STEPHEN. Usually numerous local lesions develop on carborundum-dusted tobacco leaves rubbed with inoculum prepared by crushing streak-infected leaves of *Nicotiana rustica* with 0.1 M Na_2HPO_4, but at times the number of such lesions is small. Thorough crushing of leaves in preparation of inoculum is one factor that reduces the number of local lesions, as compared with inoculum not so thoroughly crushed. Addition of juice from healthy leaves of *N. rustica* or *N. tabacum* to inoculum prepared from infected *N. rustica* also reduces the number of local lesions produced by the inoculum. It is possible that such an inhibitor may interfere in studies on concentration or rate of multiplication of virus in infected plants. Tobacco mosaic virus is not inhibited by juice from healthy tobacco plants.

Aspects of fungicidal control of snapdragon rust. DIMOCK, A. W., AND K. F. BAKER. Injury to snapdragons (*Antirrhinum majus*) from rust (*Puccinia antirrhini*) results from (1) a comparatively benign host-parasite interaction, (2) desiccation of invaded areas, (3) invasion by secondary organisms through rust-infected tissues, the type of injury depending on moisture conditions. With high inoculum potential the rust fungus was not controlled by weekly applications of Bordeaux mixture (8–8–100 plus DuPont Spreader-Sticker 1–1000) under either moist conditions at Ithaca, New York, or semi-arid conditions at Los Angeles, California. At Ithaca, nonsprayed snapdragons were only slightly damaged by desiccation, but all leaves were killed by secondary organisms. Bordeaux, though failing to control rust, gave almost complete control of secondary organisms and thus prevented loss of foliage, so that the plants appeared vigorous. Under dry conditions principal injury is from desiccation, and secondary organisms are not involved; hence Bordeaux does not prevent plant damage. These results may explain the reported effectiveness of Bordeaux under moist conditions in England and British Columbia, and its ineffectiveness under dry conditions in the United States, Europe, and Egypt. In contrast to Bordeaux, Parzate (2 lb./100 gal., plus above spreader) gave almost perfect control of rust, and, therefore, of secondary organisms, without host injury, in both localities.

Chemotherapeutic action of 8-quinolinol benzoate against Dutch elm disease. DIMOND, ALBERT E. When treated with 8-quinolinol benzoate, Dutch elm diseased trees suffer half as much as untreated trees. Beneficial effects are most pronounced when 0.1

per cent solutions are injected under pressure into the soil around feeding roots at the rate of 5 gal. per inch DBH. Surface watering over feeding roots is less effective and scattering of dry chemical followed by watering is ineffective. Treatment before leaf buds expand is without effect; in May and June treatment results in fewer foliage symptoms than in checks; and in August and September it reduces overwinter dieback. Trees treated in very early stages of infection are benefited most; those treated after involvement has become extensive are scarcely benefited. Although remedial action starts to disappear within 90 days and wears off completely in one year, differences between treated and untreated trees may be maintained by reapplying the chemical within six months to a year. Healthy trees may possibly be protected against natural infection when inoculum potential is low. Recovery of diseased trees is infrequent, but treated ones recover more often than untreated ones do.

Injury to cotton from 2,4-D weed-killer. DUNLAP, A. A. Fields of cotton in the Gulf Coast area of Texas, apparently injured by 2,4-D dust applied to neighboring rice fields in late May and early June, were kept under observation during the remainder of the growing season. The effects of the chemical on vegetative and fruiting parts of the plants were recorded in fields planted on different dates. No cotton plants were found that had died from the effects of the chemical in the affected fields. Young plants recovered from 2,4-D injury more rapidly than old plants. Rate and degree of recovery depended on such factors as age of plants, degree of injury, rainfall, and soil fertility. Owing to the high sensitivity of cotton to 2,4-D, minute traces of the chemical apparently can cause marked morphological changes. Evidence of impaired germination of the cotton seed has been obtained.

Effects of sugar concentration on the size of conidia produced by Helminthosporium victoriae. ELLIOTT, EDWARD S. As part of a more extensive study of factors influencing fructification of fungi, the effect of the carbohydrate content of culture media upon sporulation of *Helminthosporium victoriae* M. & M. has been investigated. Isolates from Vicland oats (West Virginia) sporulated abundantly under most laboratory conditions. Conidia produced on medium containing 2 per cent sugar were approximately one-third the length and contained one-third as many cells as conidia produced on medium containing 0.1 per cent sugar. Within limits, the length of conidia and the number of septations are inversely proportional to the quantity of carbohydrate in the medium. Variation in kind and amount of nitrogen had no apparent effect upon the size of conidia. These results emphasize the need for caution in comparing spores produced in culture with those produced in nature. The diminutive conidia germinate as readily as normal sized conidia.

Investigations on the physiology and pathogenicity of Ustilago zeae. FELDMAN, A. W. A comparative physiological study was made on seven lines of *Ustilago zeae*. These lines included five haploids (17 D₄ and 10 A₄, lines of opposite sex, and three mutants of 17 D₄). The two solopathogens (410 n, a weak solopathogen, and 410 yy, a strong solopathogen) were obtained from a cross between 17 D₄ and 10 A₄. Differences among the lines were evident by: 1) pathogenicity; 2) proteolytic activity; 3) growth in liquid media; 4) effect of enzyme inhibitors; 5) effect of donators; and 6) respiration studies with seven carbohydrates, seven organic acids, and eight amino acids. Combination tests (employing manometric techniques) with lines of opposite sex as well as with the solopathogens indicated: 1) no stimulating substance(s) was formed when glucose was used as the metabolite; 2) stimulation was observed when two lines of opposite sex were combined with corn extract as the metabolite; 3) no stimulation resulted when two lines of the same sex were combined; and 4) stimulation substance(s) was obtained from culture extracts but not from galls or from the protoplasm of lines tested. It was possible to correlate the physiological activities of the lines and combinations with the pathogenicity ratings obtained by tests on corn inoculations over a two-year period.

Physiological effect of Ustilago zeae on corn. FELDMAN, A. W. The effect of *Ustilago zeae* on the host was studied by comparing the respiratory rates of the nodes from healthy and diseased plants, and of galls. The effect of a gall on the ear located at node 6 was a lowering of the respiration of all the nodes below the site of infection. No galls were produced by plants inoculated with line 410 n (a weak solopathogen) but nodal respiration was inhibited down to the fourth node. The aerobic respiration of the nodes from diseased plants was inhibited more than the anaerobic respiration of the corresponding nodes. It was possible to correlate the relative pathogenicity ratings for seven lines and combinations of *Ustilago zeae* to the Qo₂ values obtained at node 3 from plants inoculated with these lines and combinations. The R.Q. values for healthy nodes, nodes from diseased plants, and galls were 0.82–0.85, 0.68–0.73, and 1.20, respectively. Catechol and ascorbic acid increased the oxygen uptake of all three tissues. Potassium

bisulphite and sodium fluoride were without effect on any of these tissues, but malonic acid and sodium nitrite inhibited most the respiration of nodes from diseased plants. It was impossible to demonstrate any differences among the lines and combinations of *Ustilago zeae* in the physiology of the galls, or in the nodal response to inhibitors and donators.

Correlation between sugar beet crop losses and greenhouse determinations of soil infestations by Aphanomyces cochlioides. FINK, HARRY C. Soil samples were taken from 24 sugar-beet fields in the spring, prior to field planting. Treated (ethyl mercury chloride) seed was planted in these soil samples in the greenhouse. The percentage of seedlings infected in 30 days by *Aphanomyces cochlioides* was determined and recorded for each soil sample. The percentages ranged from 0 to 100. During August the loss in stand and the presence of diseased beets in each field was estimated as percentage loss of the crop. The estimates ranged from 0 to 95 per cent; three poor fields had been destroyed all or in part by growers. There was a correlation of 0.925 between percentage of seedlings killed by *A. cochlioides* in the greenhouse and field crop-loss estimates. Greenhouse determination of soil infestation by *A. cochlioides* offers promise as a means of predicting sugar-beet crop losses.

Hybridization between Ustilago hordei and U. bullata. FISCHER, GEORGE W. Several hybrids of *Ustilago hordei* × *U. bullata* were obtained, when one collection of *U. hordei* from *Elymus canadensis* and four races of *U. bullata* were crossed. These interspecific hybrids yielded F_1 spores which germinated in such bizarre fashion that F_1 spores had to be used to obtain the F_2 generation, rather than monosporidial cultures from F_1 spores. When *U. bullata* from *Bromus tectorum*, having verrucose spores with uniformly colored wall, was crossed with *U. hordei*, having smooth spores with wall lighter colored on one side, the F_1 and F_2 spores were echinulate and distinctly lighter colored on two sides. When *U. bullata* from *B. purgans*, having large (8–11 μ) verrucose spores with uniformly colored wall, was crossed with *U. hordei*, having smaller (5–8 μ), smooth spores with wall distinctly lighter-colored on one side, the F_1 spores were small (5–9 μ), minutely echinulate and with a tendency to be lighter colored on two sides. The F_2 generation segregated as follows: small (7–10 μ), echinulate, with a tendency to be lighter colored and more strongly echinulate on one side; small (6–9 μ), smooth, lighter colored on two sides; large (13–16 μ), punctate, with uniform wall; and large (11–12 μ), echinulate, with spores lighter colored and more strongly echinulate on one side.

Hybridization between Ustilago striiformis and U. bullata. FISCHER, GEORGE W. From pedigreed monosporidial cultures of opposite sex, 10 F_1 hybrids were obtained between *U. striiformis* forma *hordei* and two races of *U. bullata*, when *Agropyron trachycaulum* and *Elymus canadensis* served as common hosts. Without exception the F_1 sori were of the stripe type typical of *U. striiformis*, although in 9 of the hybrids sporulation was in the glumes and flag leaf rather than in the lower leaves. Germination of the F_1 spores was so bizarre that monosporidial cultures were not obtained and it was necessary to use F_1 spores as inoculum to obtain the F_2 generation. The percentage of infection obtained in the second generation was too low to permit the determination of genetic ratios of segregation. Three of the F_1 hybrids produced only stripe type sori in the F_2, one produced only head type, two segregated into both, and four failed to produce a second generation. The F_1 spores were minutely echinulate as compared with the prominent echinulations in *U. striiformis* and the rough verruculations in *U. bullata*. In the F_2 the head type sori contained verrucose spores, while the stripe type sori contained echinulate spores.

Host nutrition in relation to development of bacterial wilt of tomato. GALLEGLY, M. E. Bonny Best tomato plants were grown in constant drip sand cultures of 0.1, 0.5, 1, 2, and 3 times a basal salt solution and in cultures with the basal solution low and high in nitrogen, phosphorus, and potassium. Inoculations were made after 24 to 28 days' growth by dipping the washed roots in a concentrated cell suspension of *Bacterium solanacearum*. In concentrations of the balanced solution, disease development was greatest at the 0.1 level and decreased with an increase in salt concentration. Little difference was observed in the two highest solutions. In the unbalanced solution low in potassium, disease development was increased over that in the basal solution; while in the solution high in nitrogen, disease development usually was decreased. No correlation of disease development with growth of the host was observed.

Reaction of onion varieties to isolates of the pink-root organism. GORENZ, A. M., AND R. H. LARSON. Using a sand-culture seedling test, 48 lots of onion, including major standard varieties, were inoculated with isolates of *Phoma terrestris* from Louisiana,

Texas, California, Illinois, Iowa, Colorado, Utah, Massachusetts, and Wisconsin. Yellow Bermuda, Beltsville Bunching, and one lot of White Sweet Spanish were the most resistant. Crystal Grano, Ailsa Craig, and Autumn Queen were the most susceptible. In reaction to any given isolate, the varieties always assumed the same relation to one another in order of resistance and susceptibility, although some isolates were highly virulent and others mildly so. With the three most virulent isolates, one each from California, Colorado, and Louisiana, the Yellow Bermuda, Beltsville Bunching, and White Sweet Spanish seedlings were nearly all killed in the course of the test but nevertheless succumbed more slowly than the more susceptible varieties.

Investigation on the mechanism of fungicidal activity. GOTTLIEB, DAVID, AND DAVID DAVIS. Six materials were investigated to determine their effect on the respiration and growth of *Sclerotinia fructicola.* The fungicides were used at concentrations of 0.1, 1.0, 10, 100, and 1000 p.p.m. in Blakeslee's 230 medium. Growth was determined after a 48-hour period and respiration was measured in a Warburg manometer for three hours after the addition of the compound. The effects of all compounds were not the same, since growth was more readily inhibited than was respiration with some of the materials but not with others. Thus 8-quinolinol sulfate and copper-8-quinolinolate inhibited growth almost completely at concentrations as low as 0.1 p.p.m. whereas no inhibition of respiration could be measured with 10 p.p.m. No inhibitory effects were observed with 5,7-dinitro-8-quinolinol at any of the concentrations used. Isothan Q15 both prevented growth and inhibited respiration almost completely at 10 p.p.m! Copper sulfate $5H_2O$ exerted no deleterious effect on either growth or respiration in Blakeslee's medium until 1000 p.p.m., but when the respiration of washed mycelium in a 4 per cent glucose solution was measured, inhibition of respiration was observed at 1.0 p.p.m. Variable results were obtained with 2,4-dinitrophenol.

Preliminary observations and experimentation on relation of insects to purple-top of potatoes in Minnesota. GRANOVSKY, A. A., AND A. G. PETERSON. Purple-top wilt of potato, occasionally serious in Minnesota, was of minor importance in 1947. The symptoms of purple-top wilt, as occurred in Minnesota are differentiated from similar symptoms associated with other diseases. Although the symptoms agree with those reported by Leach for purple-top wilt, the definite cause of the disease has not been verified. During 1947, purpling of foliage in most fields was the result of rhizoctonia stem cankers and blackleg infections rather than purple-top wilt. Usually purpling symptoms caused by these other diseases are attributed to purple-top wilt. Efforts to produce typical purple-top wilt on potatoes in outdoor cages and greenhouse have thus far been unsuccessful. Insects experimentally used: *Macrosteles divisus* from aster-yellows-infected asters; from barley; and from wild host plants; also such insects as *Polyamia inimica, Adelphocorus lineolatus, A. rapidus, Lygus oblineatus, Miris dolabratus, Stenotus binotatus,* and *Capsus atratus.* Periodic insect surveys of potato fields were made in various sections of the State during the season. The only appreciable differences in insect and disease occurrence in so-called purple-top areas have been greater abundance of *Lygus* and *Adelphocorus* spp. and increased incidence of aster-yellows on carrots without increased populations of *M. divisus.*

A pod-distorting strain of the yellow-bean mosaic virus. GROGAN, RAY G. In 1946 bean plants of Refugee varieties highly resistant to *bean virus 1* were found in Wisconsin fields, which showed leaf symptoms of yellow bean mosaic (*bean virus 2*), but were more severely stunted than typical, and bore severely distorted pods. The common strain of *bean virus 2* infected all varieties tested, causing stunting and chlorotic mottling of the leaves. The virus from pod-distorted plants did not infect Great Northern UI 59, UI 123, and UI 81. It caused top necrosis on Great Northern UI 1 and UI 15, Michelite, Stringless Blue Lake, Scotia, Tendergreen, Bountiful, Potomac, and McCaslan, and its host range was much narrower than that of the common strain. Its physical properties were: heat inactivation, 58–60° C.; longevity *in vitro,* 32 hours; dilution end point, 1–2000. Richards and Burkholder's strain of *bean virus 1* and the common strain of *bean virus 2* both protected against top necrosis on Michelite, while southern bean mosaic (*bean virus 4*) and Whipple and Walker's strain 14 of *cucumber virus 1* gave no protection. The pod-distorting virus is considered a strain of *bean virus 2* and as being related to Richards and Burkholder's strain of *bean virus 1.*

The relation of common bean mosaic to black root. GROGAN, RAY G. Certain varieties of string bean derive their high resistance to common mosaic and greasy pod from Corbett Refugee. Ordinarily, plants of these varieties show no symptoms when inoculated with *bean virus 1,* and the virus is not recovered from such plants. It was noted that rare plants, inoculated by rubbing with the virus of common mosaic or that of greasy pod, developed extreme necrosis symptoms. When plants were inoculated by

approach graft with susceptible Stringless Green Refugee plants (which were inoculated after the graft union took place) a high percentage of plants developed necrosis similar to that described for black root. When plants were inoculated by hypodermic injection similar results were secured. If plants were so inoculated in the early-pod stage, necrosis of pods characteristic of black root followed. It is concluded that black root is an expression of common bean mosaic by plants that are highly resistant to the virus. Varieties of bean, deriving their resistance from Robust or Great Northern varieties, and in which resistance is distinct genetically from that of Corbett Refugee, showed no necrosis or black root when inoculated. All bush-bean varieties tested that were tolerant or susceptible to *bean virus 1* remained free from black root.

Apple rust controlled by airborne application of Fermate. GROVES, A. B. Seven-acre plots of thirty-odd-year-old York and Winesap apple trees received airborne applications of spray concentrates beginning with the pink spray and continuing through the first cover spray when applications were discontinued after a freeze-out. Fungicides used in the concentrate formulae included liquid lime-sulfur, Puratized Agricultural Spray, and Fermate, plus various adjuvants. Application was at the rate of 8 gallons per acre; 7¼ oz. of Fermate was used per gallon. A single Fermate application made on May 11 reduced foliage infection from an average of 17.94 lesions per leaf on the poorest plot to 1.05 per leaf, a reduction of over 94 per cent. The application followed a favorable rust infection period of six days duration by four days, and was followed by another period of two days duration after two days.

Compatibility of organic fungicides with acaricides and DDT. GROVES, A. B. Combinations of organic fungicides plus DDT with the acaricides DN–111, Compound 714, Genicide, and hexaethyltetraphosphate were applied to Delicious, Grimes, and York apples to determine their compatibility in terms of fungicidal effectiveness and safety. Fermate and Bioquin gave fair scab control and were unaffected by all acaricides except Genicide, which added to their effectiveness. Phygon and Compound 341 gave good scab control and were unaffected by combinations. Scab control with Puratized was poor and variable, its effectiveness being reduced by hexaethyltetraphosphate and fortified by Genicide. Fermate and Phygon gave excellent rust control in all combinations. Puratized gave fair rust control, Genicide increased its effectiveness while both hexaethyltetraphosphate and Compound 714 destroyed its value against rust. Bioquin was ineffective against rust and although Compound 714 increased its effectiveness some, best results with Bioquin were obtained when used with DN–111 or Genicide. Compound 341 gave fair rust control and was unaffected by all combinations. All organic fungicides tested proved non-injurious when used with DDT alone. All combinations with Genicide were unsafe, that with Compound 341 causing the least injury. Phygon plus DN–111 caused fruit and foliage injury. Fruit set was too variable to secure complete data.

Three viruses of canning pea. HAGEDORN, D. J. Three apparently undescribed viruses of canning pea, *Pisum sativum*, were isolated from plants in Wisconsin pea fields. One virus produced mottle of the leaves; another caused streaking of the stem; the third brought about stunting of the plant. Host range tests indicated that all three viruses are limited to the Leguminosae. The mosaic and stunt viruses were transmitted by pea aphid (*Illinoia pisi*), but attempts to transfer the streak virus by this means yielded negative results. The mosaic virus was infective to *Melilotus alba, M. officinalis, Trifolium pratense, T. incarnatum, Phaseolus vulgaris, Vicia faba, Lathyrus hirsutus, Trigonella foenum-graecum*, and *Pisum sativum* var. *arvense*. It was not transmitted through seeds collected from diseased peas or beans. It tolerated dilution of 1:10,000 and was inactivated *in vitro* within 24 hours. The streak virus was infective to *M. alba, Trifolium pratense, T. incarnatum, T. hybridum*, and *L. hirsutus*. It tolerated dilution of 1:1,000,000 and remained active *in vitro* for seven days. Hosts of the stunt virus included *T. pratense, T. incarnatum, Trigonella foenum-graecum*. It was slightly infectious at 1:10,000 dilution and was inactivated *in vitro* at about 48 hours.

Cure of crown gall with antibiotics. HAMPTON, JACK E. Through the use of penicillin and streptomycin, both commercial and locally produced crude, numerous cures of crown gall have resulted. Usually the galls were treated by applying to them a cotton-wool pad saturated with the antibiotic. Streptomycin is somewhat more efficient than penicillin, but more than one treatment with either has seldom been necessary. The antibiotics have also been used in immersions of galled roots and by hypodermic injections. Treated galls have been cured on *Bryophyllum pinnatum, Lycopersicon, esculentum, Ricinus communis, Prunus domestica* (Green Gage Plum), *P. salicina* (Satsuma Plum), *Pyrus communis* (Bartlett Pear), *Prunus cerasus* (Duke Cherry), *Ligustrum lucidum*, and other plants. Galls treated on these plants were either aerial or sub-

terranean, soft or hard, on the stems, taproots, and secondary roots. Use of antibiotics on crown gall should prove particularly valuable in nurseries.

Victoria blight of oats in Kansas. HANSING, E. D., HURLEY FELLOWS, C. O. JOHNSTON, AND A. L. CLAPP. In 1946 Victoria blight caused a loss of 1 per cent. In 1947 the disease was generally distributed on susceptible varieties and caused a loss of 30 per cent in northeastern Kansas, 20 per cent in southeastern Kansas, 5 per cent in central Kansas, and a trace in western Kansas. Osage, Neosho, Boone, and Tama were susceptible, while Kanota and Fulton were resistant. Cherokee, Nemaha, and Clinton were recommended for 1948. These are resistant to Victoria blight, smut, and the crown and stem rust races found commonly in Kansas. Victoria blight was favored by high soil temperatures (optimum 22° to 26° C.) and by moderately high moisture. No symptoms of the disease were observed on Neosho oats grown in soil at 10° and 13° C., but when these plants were transferred to 23° C. the disease developed rapidly. Seed treatment was effective in controlling the disease in sterile soil, but only partially effective in infested soil. Crop rotation was partially effective in controlling Victoria blight. In eastern Kansas, when susceptible oat varieties were preceded by the following crops, percentages of loss occurred as follows: susceptible oat varieties, 38; corn, 27; clover and meadow, 26; soybeans, 24; wheat, 18; and resistant oats, 4.

Field inoculation technique with Aphanomyces root rot of garden pea. HARE, WOODROW W., AND DARRELL G. WELLS. Natural infection by the root rot organism, *Aphanomyces euteiches* Drechsler, is extremely variable from year to year in Wisconsin. To test possibilities of artificial inoculation of field plots two varieties of garden pea (*Pisum sativum* L.) were planted in 1947 according to a split-plot design. The Pride variety, because of observations indicating possible tolerance to the disease, was compared to highly susceptible Wisconsin Perfection. Three applications of inoculum of *A. euteiches* were placed on separate rows at different times with one row left as check. Plants were classified according to disease reaction 76 days after planting, and disease indices were calculated. There were no significant differences between varieties or interaction of varieties and treatment. Differences between treatments were highly significant. Inoculations made before planting and when the peas were 6 to 8 inches high produced significantly more disease than inoculation when the peas were in late blossom. Late blossom inoculation was significant over the check.

Stem rust of wheat in Mexico. HARRAR, J. G., AND N. E. BORLAUG. As previously reported, the tendency for the establishment of all of the important physiologic races of *Puccinia graminis tritici* in all wheat-growing areas of Mexico is increasingly evident. Race 38 is regularly found in both northern and central Mexico although not reported from Sonora in the northwestern part of the country. Race 17 is found in all three regions as is race 56. Races 59, 59–A, and 19 are still restricted to central Mexico. It is probably not feasible to consider that varieties may be selected for the several wheat-growing areas of Mexico on the basis of their resistance to predominant races found in those regions, but rather is it necessary to consider that improved wheats for Mexico must be developed with reference to all the important races found in the country. At the present time there are available for increase, selections from varieties of Kenya, Trigo Supreme × 41–116, and Frontaira × 41–116, and selections from crosses of Newthatch × Marroqui, Kenya × Aguilera, and Mida × Pelón Colorado, which in test give promise of a high degree of resistance to the predominant physiologic races found in Mexico.

Resistance in onion to smudge, neck rot, and black mold. HATFIELD, W. C., AND J. H. OWEN. Twenty-two varieties of onions were tested in an attempt to correlate disease incidence with certain characteristics of the varieties. Using a bioassay, little correlation was found between the fungicidal and fungistatic action of the expressed juice and its vapor and disease incidence of smudge (*Colletotrichum circinans*). A definite correlation existed between certain scale characters and smudge incidence in colored varieties. Those varieties with poor outer scale adherence were more susceptible than those with tight, well developed scales. This does not apply to the white varieties where absence of color leads to susceptibility. The incidence of neck rot (*Botrytis allii*) was definitely correlated with pungency, those varieties highly pungent having lower disease incidence than the milder varieties. Incidence of black mold (*Aspergillus niger*) was correlated with bulb color differing from smudge in that colored varieties were more susceptible than the white ones. The parasitic action of the three organisms varied with their tolerance to the antibiotic substances present in expressed juice of the succulent scales. *B. allii* was the most aggressive and the least affected by the antibiotic substances. *C. circinans* was less aggressive and more affected. *A. niger* was least aggressive and most affected by the antibiotic substances.

Pathogenicity of single spore isolates of the fusarium-wilt organisms of muskmelon and watermelon. HENDRIX, J. W., E. P. DU CHARME, AND HARRY MURAKISHI. Previous workers have attributed watermelon wilt and muskmelon wilt to forms 1 and 2 of *Fusarium bulbigenum* var. *niveum*, respectively, and have found no exceptions to strict host-pathogen specificity. Further research by the writers has shown that some isolates from wilted muskmelon plants cause severe pre-emergence killing and wilting of watermelon seedlings in the greenhouse. Some isolates from watermelon parasitize muskmelon. For example, certain monosporous isolates from muskmelon caused as much as 40 per cent reduction in stand of watermelon while similar losses resulted when muskmelon seedlings were grown in soil infested with isolates from wilted watermelon plants. On the original host plants, different isolates caused from 0 to 100 per cent killing. In general, the virulence of an isolate was greater on its original host than on the second host plant; nevertheless, several isolates from watermelon caused a more severe reduction in stands of muskmelon than of watermelon.

Wilt epidemiology and resistance in the mimosa tree. HEPTING, GEORGE H., AND E. RICHARD TOOLE. Within the 12 years since the fusarium wilt of *Albizzia* was discovered, it has spread widely until now it is known in 76 counties from Maryland to Alabama. At Morganton, North Carolina, where the wilt appeared on one city block in 1943, trees were dead or dying on 232 blocks by 1947. Search for wilt-resistant mimosas was begun in 1939. Six hundred and thirty-two seedlings were grown from seed collected from Maryland to Louisiana. After several inoculations, the remaining seedlings were planted in infested soil. Twenty of them have remained wilt-free for 5 years, while neighboring volunteers have died. Eight stem cuttings rooted from these trees and 17 from some random volunteers were inoculated at one time. All volunteer cuttings died of wilt the first year, but none of the cuttings from the resistant selections died. Rooted cuttings from trees selected at random from other locations have also proved to be susceptible. Thus, certain individual mimosa trees appear to be wilt resistant.

The dithiocarbamate fungicides and the control of downy mildew disease of cucumbers and cantaloupes. HEUBERGER, J. W., AND L. P. NICHOLS. Research during 1946 and 1947 on the use of Zerlate (zinc dimethyl dithiocarbamate), Fermate (ferric dimethyl dithiocarbamate), Dithane Z–78 and Parzate (zinc ethylene bisdithiocarbamate), Dithane D–14 + zinc sulphate–lime (zinc ethylene bisdithiocarbamate), and manganese ethylene bisdithiocarbamate as sprays at 1½–100 active ingredient concentration for the control of downy mildew on cucumbers (pickles) and cantaloupes, has shown the following. *Cucumbers:* Zerlate, Parzate, and Dithane Z–78 were as effective in control as Bordeaux and the fixed coppers, increased early and total yields more than the copper compounds, and were noninjurious when properly formulated; Dithane D–14 + zinc sulphate–lime was not so effective as Zerlate, Parzate, and Dithane Z–78; Fermate was injurious under certain conditions (hot, dry weather); manganese ethylene bisdithiocarbamate was injurious. Yield increases from Zerlate, Parzate, and Dithane Z–78 were respectively, 48, 29, and 50 per cent in 1946 and 54, 81, and 71 per cent in 1947. *Cantaloupes:* Zerlate, Dithane Z–78, Parzate, and Dithane D–14 + zinc sulphate–lime were equal in control to Bordeaux and the fixed coppers and gave higher yields. Fermate and manganese ethylene bisdithiocarbamate were injurious. When similar materials were compared as sprays and dusts in 1947, the dusts give higher yields under the hot, dry weather conditions prevailing in late July and August.

Induced wilting of tomato cuttings by synthetic polymers of varying molecular weight. HODGSON, ROLAND, W. H. PETERSON, AND A. J. RIKER. Studies with certain toxic water-soluble polysaccharides have suggested a direct relationship between the molecular weight (range 1,000 to 5,000) and the wilt-inducing potency on tomato cuttings. Such a relationship was sought with other water-soluble polymers available in several molecular-weight ranges. Solid polyethylene glycols (0.2 to 0.4 per cent aqueous solutions) induced severe wilting and necrosis of leaflets; the symptoms varied somewhat with the different compounds but were similar to those caused by a glucosan from crown-gall bacteria and certain other polysaccharides. A direct relationship between molecular weight (range approximately 1,500 to 9,000) and wilt-inducing activity was found, the largest being most toxic. This suggests that the wilting mechanism may be mostly physical. Evidence for an accumulation of the toxic agent in or near the injured areas was sought by analyses of cuttings treated with polyethylene glycols. Of the glycol taken up, 87 to 97 per cent was recovered; of this, 81 to 91 per cent was found in the leaflets. Wilted portions of leaflets contained more glycol than non-wilted portions, showing a greater accumulation in the region of the injury.

A new type of resistance to spotted wilt. HOLMES, FRANCIS O. Spotted-wilt virus isolated from a recent outbreak of disease in dahlia and tomato in New Jersey was found

to be capable of overcoming the characteristic resistance of the Pearl Harbor tomato, which is adequate for protection from spotted wilt in the Hawaiian Islands. A new and locally applicable type of resistance was found in a tomato derived from Argentina. This new type of resistance proved effective both in greenhouse and field tests. It showed monofactorial inheritance in hybrids with the susceptible variety Rutgers. The new type of resistance together with the only formerly known type, that in the Pearl Harbor tomato, may be expected to serve a dual purpose, of facilitating control of the disease and of indicating the geographical distribution of differing strains of spotted-wilt virus.

Evidence of parasitic activity of Actinomyces scabies on seedling roots. HOOKER, W. J., AND J. E. SASS. It was previously reported that seedling roots of a number of plants developed severe necrosis when grown in soil-water agar artificially infested with *Actinomyces scabies* whereas roots of the same species showed no necrosis with cultures nonparasitic to potato. Necrosis of a comparable type, although less severe, developed on soybean and wheat roots grown in quartz sand artificially infested with *A. scabies*. There was no evidence that such necrosis was produced by a water soluble substance secreted by *A. scabies* on potato-dextrose agar. Sectioning demonstrated *A. scabies* filaments abundant in the rhizosphere and in the epidermal and cortical cells of these roots grown in agar even though the root was not noticeably necrotic. *Actinomyces* spp. were demonstrated by dilution plate counts to be appreciably more abundant in the rhizosphere of field-grown oat roots than in soil in which there were no roots. Roots of Clinton oat plants grown in the peat soil in northern Iowa were sectioned and the filaments of an actinomycete were demonstrated in the tissue.

Some histological features of potato stem necrosis associated with Actinomyces scabies. HOOKER, W. J., AND J. E. SASS. Of the varieties of potato tested to date, those known to be susceptible to tuber scab (*Actinomyces scabies*) exhibited considerable necrosis of stems resulting from infection by the scab organism, whereas varieties resistant to tuber scab were likewise resistant to stem necrosis. When plants were grown in the greenhouse in sterilized peat soil artificially infested with *A. scabies*, infection was often established in the stems through unwounded surfaces away from stolons and secondary roots. In this type of lesion, the susceptible varieties, Red Warba, Cobbler, Pawnee, Katahdin, and Chippewa, showed either no periderm or at most a poorly developed, discontinuous periderm, with actinomycetous filaments abundant in the cortical cells. The stems of the resistant varieties, Menominee, Cayuga, and two unnamed clones, showed well-developed and continuous periderm with sparse intracellular mycelium. This relationship did not hold consistently with resistant and susceptible varieties naturally infected in the field. Where stem infection in the field took place through natural wounds such as longitudinal splits or at points of emergence of stolons and secondary roots, periderm formation was generally poor, even in resistant varieties, and mycelium was often abundant.

A spiral design for the field assay of pesticides. HORSFALL, JAMES G., SAUL RICH, AND NEELY TURNER. Single-row plots are arranged in a continuous spiral row. Planting is simplified by attaching the planter or marker to a cable that winds around a fixed vertical drum anchored at the center. The planter progresses inwardly at a uniform rate allowing three-foot intervals between laterally adjacent plots. A roadway is left for placing the trailer-mounted power sprayer at the spiral center, near the water supply and drainage pipes. An overhead, rotatable, horizontal aluminum pipe carries the spray material to a length of rubber hose attached to a hand carried, three-nozzle spray boom. The sprayer has two tanks for simultaneous spraying and mixing. Ten-foot plots of nonstraggling plants are randomized in each quadrant, making four replicated wedge-shaped blocks. The hand-carried spray boom is flexible, the power pump untiring; the circular route of travel saves a return empty trip; and the water supply and drainage arrangements save work and time in loading. Drift error is negligible. Spring-loaded check valves, ahead of the spray nozzles, prevent drizzle between plots. Inoculation is aided by a rotating overhead irrigator, controlled at night by a timing apparatus.

Root-rot complexes of tobacco and small grains in Virginia. JENKINS, WILBERT A. Studies of several root rots of tobacco and cereals in Virginia offer proof that feeder root mutilation is a primary factor in initiation of the diseases. Feeder root mutilation is accomplished through the feeding habits of various members of the meadow nematode group. Representatives of two nematode genera, *Pratylenchus* and *Ditylenchus,* cause primary pathologic symptoms in addition to serious mutilation while several representatives of other genera appear principally to open up the tissues to invasion by fungus and bacterial soil associates. These studies have reference to brown and black root rot of tobacco as well as certain unclassified troubles symptomatically characterized by stunting,

malnutrition, and low production. The studies on small grains included "winter" injury of wheat and barley and certain unclassified diseases of wheat, barley, and oats whose principal symptoms were stunting, foliage bronzing, and unproductiveness. Such factors as relatively low soil temperatures and relatively high calcium levels distinctly favored nematode population increase and severity of feeder root mutilation of all groups. Higher soil temperatures and moisture in the later season definitely favored root decays, depending on the specificity of the bacterial and fungus associates in the rhizosphere.

Strains of flue-cured tobacco resistant to black shank (Phytophthora parasitica var. nicotianae) and tolerant to certain root-rot complexes. JENKINS, WILBERT A., Crosses were made in 1941 between two resistant tobaccos and a strain of Yellow Special. The resistant parent lines were developed in North Carolina from a cross between Florida 301 × Warne, with multiple backcrosses to Warne (later released as Oxford 4) and a cross between Florida 301 × White Stem Orinoco, with multiple backcrosses to W.S.O. (later released as Oxford 3). The resistant parents were about 80 per cent resistant to black shank, but low in quality and yield and susceptible to root-rot complexes prevalent in Virginia. The Yellow Special parent was susceptible to black shank, but of superior quality and yield and highly tolerant to several common root-rot complexes. Following three seasons of mass selection and three seasons of pure-line selections from progenies of these crosses, selected lines have been fixed for type. These are 95–98 per cent resistant to black shank under field conditions, of excellent quality and yield, and highly tolerant to several of the root-rot complexes found in Virginia. Throughout the period of selection, test-plot soils were heavily infested with black shank and each individual plant was inoculated with black shank material each season. Available at present are strains representing 4 leaf types. All strains were evaluated in Virginia and North Carolina last season.

Increase of virus infectivity by certain chemicals. JOHNSON, JAMES, AND PHOEBE A. GREEN. In 1935 Stanley listed 17 chemicals that increased the infectivity of the ordinary tobacco-mosaic virus. We have tested these chemicals by somewhat modified methods. The sensitive *tabacum-glutinosa* hybrid was used as a local-lesion test plant. Inoculations with the virus-chemical mixtures were made at once and after different intervals of storage. The infectivity with five chemical treatments, involving 87,324 local lesion counts with controls based at 100, were: sodium nitrate, 190; calcium chloride, 166; dipotassium phosphate, 138; sodium thiosulphate, 121; diethyl acetate, 118. The highest increase (226) was obtained with sodium nitrate after immediate inoculation. Increases as high as some reported by Stanley were not obtained. The other chemicals listed by Stanley either did not yield increases or they were too small in amount to be significant. The increased infectivity declined very slowly in storage and was retained for as long as 30 months at dilutions of 1–1000, suggesting some "preservative" value of the chemicals mentioned. The evidence secured did not indicate that increased infectivity is due to a change in hydrogen-ion concentration. It seems more likely that these chemicals favor a higher dispersion of the virus particles in the inoculum.

A yellow-mosaic virus associated with Eryngium mosaic. JOHNSON, JAMES, AND EDITH M. HEIN. Further studies have been made on a yellow-mosaic virus from a complex in *Eryngium aquaticum* L. This component produces a uniform chlorosis over older leaves of Havana tobacco plants, with occasional broad chlorotic spots or patterns on younger leaves. It is difficult to separate this virus or strain from the Eryngium-mosaic virus, although the latter may be readily freed of the yellow type. Property determinations indicate that the yellow-mosaic virus is somewhat less tolerant than is the Eryngium-mosaic virus, whose properties are similar to those of *Cucumber virus 1*. Furthermore, the yellow-mosaic virus was not transmitted by the peach aphid and did not yield symptoms on tomato, which is susceptible to the Eryngium-mosaic virus. The best differential host for the yellow Eryngium-mosaic virus appears to be White Burley (var. Judy's Pride) tobacco. On this host, inoculative and systemic chlorotic spots and necrotic lesions occur which, by repeated transfer, are helpful in freeing the virus from the Eryngium-mosaic virus. The chief interest in yellow-mosaic viruses centers around their origin and relationship to other viruses with which they are normally associated. Their ability to destroy chlorophyll or to interfere with its normal production is characteristic and significant.

A new virus disease of Ladino clover. KREITLOW, K. W., AND W. C. PRICE. A virus disease of Ladino clover (*Trifolium repens* var. *giganteum*) characterized by yellow mottling of leaves and retardation of growth, occurs extensively in the Northeastern United States. The disease is caused by a virus which is readily transmitted mechanically

to healthy Ladino clover. The virus was also transmitted to other species of *Trifolium*, to several species of *Melilotus*, and to *Pisum sativum, Phaseolus vulgaris, P. lunatus, Soja max, Zinnia elegans, Antirrhinum majus, Capsicum frutescens, Petunia hybrida, Nicotiana tabacum, N. glutinosa, N. rustica,* and *Vinca rosea.* Virus was recovered from each of these species. Typical chlorotic areas were produced in the leaves of many of the hosts tested. Symptoms in other hosts consisted of extensive mottling, downward curling of leaves, and severe stunting. Reddish-brown local lesions were produced within 48 hours when juice from infected plants was rubbed on leaves of *Phaseolus vulgaris.* The host range and properties of the virus resemble those of alfalfa mosaic virus but the symptoms are different from any known strain of the latter.

Autogenous necrosis in interspecific tomato hybrids and its relation to the breeding of tomatoes for resistance to Cladosporium fulvum. LANGFORD, ARTHUR N. Following crosses between the red currant tomato, *Lycopersicon pimpinellifolium,* and varieties of the cultivated tomato, *L. esculentum,* immune *esculentum*-like hybrids normally develop a severe autogenous necrosis, manifest chiefly in leaf-spotting, unless, along with Cf_{pl}, the dominant *pimpinellifolium* factor *Ne* is also incorporated in the hybrid. Susceptible plants show no necrosis, regardless of their genotype at the *ne* locus. Necrosis is due to the interaction among Cf_{pl} (located on chromosome 4), *ne* (located on chromosome 1) and, probably, other *esculentum* genes. Evidence has been obtained showing that one or more genes on chromosome 7 may, in progenies resulting from selfing, prevent the development of necrosis in immune plants of genotype *ne/ne*. Striking seasonal variations in the severity of necrosis have been observed: the artificial lowering of the incident light intensity greatly reduces the severity of necrosis during the summer. Although the discovery of races of *Cladosporium fulvum,* to which such varieties as Vetomold $(Cf_{pl}/Cf_{pl}; Ne/Ne)$ are completely susceptible, has complicated breeding programs and initiated searches for additional resistance factors, the widespread distribution of races 1 and 2 suggests that the incorporation of the factor Cf_{pl} in resistant varieties will still be desirable. The dominance of the immunity factor and of the factor conditioning freedom from necrosis make the backcross method of developing new, necrosis-free varieties a convenient one.

Greenhouse tests of an antibiotic substance as a protectant spray. LEBEN, CURT, AND G. W. KEITT. In greenhouse tests ethanol solutions of an unidentified antibiotic substance produced by a species of *Streptomyces* were effective as protectant sprays against apple scab. Under certain conditions these sprays caused marked phytotoxicity due to the ethanol. In the present work there was no injury of apple, tomato, or pea plants when the antibiotic material was carried in 25 per cent·ethanol in water or in water alone. With the more effective of these preparations,·containing 3–6 arbitrary units of the antibiotic substance per ml. as determined by plate assay with *Glomerella cingulata,* complete control of apple scab and early blight of tomato was obtained in greenhouse experiments. Artificial rain tests indicated that the active material resisted washing on apple or tomato foliage. When apple leaves were inoculated with *Venturia inaequalis* four days after application of the antibiotic spray there was some lessening in the effectiveness of control. Yields of 3–5 units of the active material per ml. of the culture filtrate have been obtained from tank fermentations.

Comparative cultural studies with Thielavia basicola and Thielaviopsis basicola. LUCAS, G. B. The ascomycetous fungus *Thielavia basicola* has been reported as the perfect stage of the tobacco black root-rot fungus *Thielaviopsis basicola.* Perithecia and ascospores of *Thielavia basicola* have been seen on diseased tobacco roots in association with chlamydospores of *Thielaviopsis basicola.* To date it has not been possible to show that either of these forms would produce the other. In the absence of such data isolates of both were obtained and grown on various media and under different conditions to see how each would react. On Richards agar minus sucrose, with filter paper as a carbon source, *Thielavia* grew rapidly, *Thielaviopsis* grew little or not at all. On carrot slices *Thielavia* grew slowly or not at all, *Thielaviopsis* grew rapidly. *Thielavia* grew on potato or carrot agar at pH 3, *Thielaviopsis* did not. *Thielavia* grew at 37° C., *Thielaviopsis* did not. Pathogenicity tests on tobacco with *Thielavia* were negative, whereas *Thielaviopsis* was consistently pathogenic. When the two forms were grown together on various media, no mating reaction was evident. These data are offered as further evidence that *Thielavia basicola* is not the perfect stage of *Thielaviopsis basicola.*

Results from co-operative trials with seed disinfectants on flax. MACHACEK, J. E. As a part of the program of investigations sponsored by the American Phytopathological Society's Seed Treatment Committee, experiments involving a comparison of the newer seed disinfectants with respect to their effect on germination and yield of flax

were carried out at 14 stations in Canada and the United States. The results showed significant increases in germination and yield at some stations but not at others. Seed disinfection was more beneficial to mechanically injured seed than to uninjured seed. Of the disinfectants tried, New Improved Ceresan, applied at the rate of 1 to 2 oz. per bushel, generally gave the best result.

Argentine rape as an artificial weed in experiments with seed disinfectants. MA-CHACEK, J. E., AND H. A. H. WALLACE. The oil crop Argentine rape (*Brassica napus* L.) was used to infest experimental plots with an artificial weed with the object of simulating the weedy conditions of the ordinary farm. In plots thus infested the yield of flax or wheat was found to be almost proportional to the rate of seeding. In weed-free plots, where yields were in general much higher, the increase in yield was not proportional to the increased rate of seeding—the yield at first rising rapidly to a peak and then falling off with further increase of seeding rate or, in some cases, remaining at the peak level. The implication of this finding is that seed disinfection, by increasing density of stand, may be more profitable under weedy conditions than under weed-free conditions, and that a count of seedlings may be more reliable than yield as an index of seed disinfectant values.

Changes induced in Sclerotinia fructicola on copper sulphate media. MADER, E. O., and C. L. SCHNEIDER. The tolerance of 5 monoascospore isolates of *Sclerotinia fructicola* to copper sulphate in potato-dextrose agar was determined. All isolates tolerated copper sulphate concentrations up to 7,000 p.p.m. Sporulation was increased at concentrations below 1,000 p.p.m. but suppressed at concentrations of 5,000 p.p.m. or higher. After having grown for 18 weeks on 5,000 p.p.m. all isolates tolerated 10,000 p.p.m. Isolates that had grown for 18 weeks on media containing 5,000 p.p.m. of copper sulphate were transferred to non-copper media and tested for copper tolerance and cultural characters at the end of 14 weeks and 42 weeks. Some cultures reverted to their parental type in appearance and had lost their acquired tolerance for copper. Other isolates, however, did not revert to the parental cultural type and maintained a high tolerance for copper sulphate. These variants differed in ability to rot fruit.

New fungicides for control of narcissus basal rot. McCLELLAN, W. D. New Improved Ceresan and 2 per cent Ceresan are both effective in controlling narcissus basal rot caused by *Fusarium oxysporum* f. *narcissi* but frequent flower injury results from their use. Hence an effective but safer treatment is desired. Five-minute after-harvest plus pre-planting dips of King Alfred narcissus bulbs either in Mersolite 8 (phenyl mercuric acetate) at the rate of one pound in 800 gallons, or in Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate), one part of the active ingredient in 10,000, in 1945 and 1946, have given control of basal rot equal to the standard New Improved Ceresan treatment but without the flower injury. Rot has not been controlled so well when the bulbs were dusted with Arasan or with Spergon but bulb yields have been about the same as yields following treatments with mercurials. Beginning with 200 bulbs (weighing 284 oz.) per treatment in 1945 the bulb yields in 1947 after two years of treatment were: New Improved Ceresan, 531.5 oz.; Arasan, 523; Spergon, 490; Tersan, 393.5; Mersolite 8, 522.5; Puratized Agricultural Spray, 435.5; and untreated, 109.5 ounces. Similar results were obtained from treatments begun in 1946. A number of other materials have given either poor control or severe flower injury.

Etiology and control of begonia bacteriosis. McDONOUGH, E. S. The leaf spot disease of begonia caused by the bacterium that has been referred to as *Xanthomonas begoniae* has not been satisfactorily controlled by modification of cultural practices when the plants were grown in large numbers under economically feasible greenhouse conditions. Studies on the etiology have made possible an efficient eradication procedure. Bacteria obtained from drops of sterile water which had been placed on diseased leaf regions were atomized on leaves of healthy Melior begonia plants, kept under conditions of high humidity. The plants were then sprayed with water. Only leaves atomized from the top showed symptoms typical of the naturally infected leaves. From this and other evidence it was assmed that infection generally took place by splashing during watering or by movement of the bacteria from leaf to leaf in contact with the rooting medium. Bordeaux mixture has controlled the disease, whereas other bactericides used have proved injurious to the host plant or have shown no superiority. Dipping the cuttings in Bordeaux and keeping the tops of the leaves covered during a season has resulted in practical elimination of the disease.

Relation between the condition of the seedling root system of corn and the grain. MEREDITH, CLIFFORD H. The seedling root system of the corn plant never attains a very

great length, so that it can usually be recovered for observation purposes. The mature seedling roots become rather woody and can be recovered even after over-wintering in the soil. A study was made of the condition of the seedling root system and the weight of grain produced by individual corn plants. One hundred plants taken at random from the field were measured to find, (1) circumference at the base of the stalk, (2) length of the primary root, (3) number of secondary roots, (4) length of the longest secondary root, and (5) weight of the grain produced. It was found that there was a relation between the yield and the length of primary and secondary roots. The cause of the poor condition of some of the seedling roots was apparently fungus attack at an early stage of growth. It appears that a plant attacked by a root pathogen at an early stage does not recover sufficiently to produce a normal yield. The results of these studies indicated that the condition of the seedling root is closely related to production of grain.

Root nodulation of Holland Jumbo strain peanut grown from seed treated with a fungicide. MILLER, LAWRENCE I. In a greenhouse test Spergon (tetrachloro parabenzo-quinone) applied at the rate of 3 ounces per 100 pounds of hand-shelled seeds had no effect on root nodulation as measured by number, type, and location of nodules. This was true both when the Spergon-treated seed was inoculated with 2 commercial preparations of *Rhizobium leguminosarum* (mixed cultures in a humus base) and when left uninocu-lated. The treatment with Spergon always preceded inoculation. The results were the same in nonsterilized and steam-sterilized soil. The root system of peanuts in the steam-sterilized soil was larger than in the nonsterilized soil. Nodules appeared on plants in both types of soil without inoculation, but nodules on plants from noninoculated seed were larger and fewer than on roots of plants from inoculated seed. The distribution of nodules also was different.

Factors affecting the development of Melampsora lini. MISRA, A. P. *Melampsora lini* developed best at mean temperatures of 65–75° F. in the field in 1946 and 1947, but was drastically suppressed when the maximum temperature reached 92° F. or more for two or three days. Plants of Redwing and Bison flax were inoculated in the greenhouse with race 4 of *M. lini* and then exposed for 30 hours to 95–99° F. after prominent rust flecks had developed; little or no rust developed. Most urediospores collected from the field when temperatures were 98–100° F. had lost their viability and did not germinate even under optimum conditions. Partial shading of the plants in the field at 98–100° F. did not enable urediospores to retain viability; but with partial shading at 88–90° F. from 23 to 35 per cent of the urediospores remained viable. Teliospores developed well and germinated readily at 65–75° F. but not at 85° F. Ammonium nitrate fertilizer applied alone or with potash and phosphate to sandy soils greatly increased the severity of rust, whereas potash and phosphate alone did not. Teliospores collected on Koto flax at Morris, Minnesota, in August, 1946, first germinated in January, 1947. Teliospores collected on Bison at St. Paul, Minnesota, in August, 1946, did not germinate until May, 1947. Germ tubes of urediospores of race 17A grew faster than those of race 4.

Parasitic races of Ustilago tritici on spring wheats. MOORE, M. B. Nine parasitic races of *Ustilago tritici* have been found that primarily attack common wheats, and two have been found that primarily attack durums. These races were identified on the basis of the pathogenicity for 12 varieties of spring wheat, including two durums. Some varieties, such as Hope, Vernal, and *Triticum timopheevi*, are extremely resistant to all races tried; some varieties are immune from certain races and highly susceptible to others. The durums, generally resistant in nature, are very susceptible to one or both durum races when artificially inoculated. There apparently are various types of resistance, operating singly or in combinations. A Thatcher × Supriza cross and two lines from Hope × (*T. timopheevi* × Steinwedel) have been resistant when inoculated with mixtures of 4 and of 5 races, all of which were different. The variety Cadet was found to comprise two or more morphologically similar strains, one of which is resistant to several smut races that can attack the other strain.

Specificity of certain Bioquin (8-quinolinol) derivatives and some of their formu-lations for apple-scab control. MORGAN, OMAR D., JR., AND DWIGHT POWELL. Laboratory tests of 43 Bioquin derivatives and their formulations indicated the following as fungi-cides: Bioquin 1 (copper 8-quinolinolate) and its dry mixed formulations with Santocel, Celite 266, Pyrax ABB, Wyodek bentonite, Palmetto sulfur (325 mesh), and Dowmike sulfur; Bioquin 100 (zinc 8-quinolinolate) and Bioquin 300 (magnesium 8-quinolinolate) and their formulations with pyrax, bentonite, and celite. Spray tests on Golden Delicious made with Bioquin 1 (1, ½, ¼ and ⅛–100), Bioquin 1 formulated with pyrax and with bentonite (25, 50 and 75 per cent Bioquin 1) at 1–100, indicated that under severe disease conditions the amount of control on the fruit obtained with the formulations of

Bioquin 1 with pyrax and with bentonite is significantly higher than when Bioquin 1 at ¾–100 is used alone. In the same test, Bioquin 100 and its formulations with pyrax and bentonite and Bioquin 300 gave slight control of apple scab. Tests on Jonathan included Bioquin 1 (1 lb.), Bioquin 1 (¼ lb.) plus Factice ¼ lb., Bioquin 1 (¼ lb.) plus Micronized sulfur 3 lb., Puratized 1 pint, Microsul 8 lb., Phygon ¼ lb., Bioquin 1 (25 per cent) plus Santocel (75 per cent) 1 lb. and Fermate 1 lb., each in 100 gallons of water. Of these, the first five gave over 95 per cent control on the fruit two months after the last application. By harvest time, the residual effect had been reduced; Puratized showed best control (72 per cent), Microsul (66 per cent), Bioquin 1 plus sulfur (63 per cent), and Bioquin 1 (62 per cent). The control of other materials ranged from 55 per cent for Bioquin 1 plus Factice to 8 per cent for Bioquin 1 plus Santocel. This last formulation gave best control in laboratory tests.

Nitrodithioacetates as potato sprays. MUNCIE, J. H., AND W. F. MOROFSKY. Potato-spray trials on the Menominee variety of potato were made by application of the nitro-dithioacetates of zinc and copper and copper nitroacetate, singly and in combination, in comparison with tribasic copper sulphate and Bordeaux mixture. DDT and benzene hexachloride were added as insecticides. There was no significant difference in control of early blight. Late blight was absent. Highest yield was obtained from plots sprayed with a nitrodithioacetate of copper plus DDT. Substitution of benzine hexachloride for DDT resulted in increased infestation of potato leaf hoppers and the six-spotted leaf hoppers.

An unusual host response to certain paired monosporidial cultures of Ustilago zeae. MUNNECKE, DONALD E. Certain paired monosporidial isolates of *Ustilago zeae* produced unusual leaf curlings and distortions when hypodermically inoculated into a selfed line of corn. Stem and leaf tissues remained tightly curled, but as they grew they became gnarled, twisted, and distorted; or the leaf tip remained within the sheath and a single large loop of curled leaves resulted from the growth of basal leaf tissue. The distortions resembled immature smut galls, except that they remained green and seldom were hypertrophied. Chlamydospores never were formed, but smut mycelium was recovered when some of the distorted tissues were plated on potato-dextrose agar. In four green-house tests extending over six months three pairs of white cultures caused this pronounced leaf curling without resultant chlamydospore formation. The distortions were not due to mechanical injury caused by the hypodermic needle, because sterile broth check inoculations were symptomless.

Onion-mildew control in 1947. NELSON, RAY. Airplane dusting with copper-sulphur fungicides was not effective against onion downy mildew in the 1945 epidemic. Mildew did not reappear in 1946 and only yield data were obtained on effects of fungicides. Dusts and sprays were applied in 1947 to seeded Yellow Globe onions in a randomized block design with individual plots of four 30-ft rows. The first application July 7 pre-ceded mildew detection, but all plants in the untreated plots were infected at the time of the fifth and final application on August 17. Dusts were applied at 50 lb. and sprays at 204–272 gal. per acre. DDT supplemented all materials for thrips control and Triton–1956–B as detergent in all sprays. Spray treatments were: Cuprocide; Cuprocide–p.e.p.s; Cuprocide–Dowax; Cuprocide–wettable sulphur; Dithane HE–178; Dithane HE–178–p.e.p.s; Dithane HE–178–Dowax; Bordeaux; Bordeaux–Dowax; P.e.p.s.; DDT; Wettable sulphur; Dow 606 (Zn-dimethyl-dithiocarbamate); Dow 608 (amine salt tetra-chlorphenol); Dow 612 (1,4-dithio-cyano-butene); Lime-sulphur-rosin. Dusts were: Cuprocide-sulphur and Dithane HE 178. Effective mildew control with no apparent phytotoxicity was obtained with both dusts and with Dithane, Dow 612, and Bordeaux sprays. Cuprocide sprays controlled mildew but caused some injury. Significant yield increases from mildew control alone were: Dithane dust, 29 per cent; Cuprocide dust, 21 per cent; Dithane HE 178, 17 per cent; Dow 612, 12 per cent; and Bordeaux, 8 per cent.

Field trials in New York with ethylene dibromide and DD mixture against the root-knot nematode. NEWHALL, A. G., AND B. LEAR. With the aid of a tractor-drawn, three-row, sub-surface applicator, loaned and operated by the Shell Chemical Corporation, dosage rates of 200 and 300 pounds per acre of DD mixture and a 10 per cent ethylene dibromide mixture were compared for the control of root-knot nematode when applied in rows 10 and 12 inches apart in muck and upland soils. Weeds were not controlled to any extent by any treatment. Stands of carrots showed that the dosages used were near the maximum limit for spring treatments because of possible injury to seedlings and possible effect on flavor of carrots. Three tests conducted on muck soil showed a 50.9 per cent increase in yields of No. 1 carrots over checks for the DD treatments and 49.4 per cent increase for the ethylene dibromide treatments. Tests on two upland plots

showed a 155 per cent increase for DD mixture and a 122.8 per cent increase for ethylene dibromide. The data showed that the 300-lb. dosages were better than the 200-lb. dosages with both materials and application of the materials in rows 10 inches apart more effective than at the 12-inch distance.

Studies on genetics of Ceratostomella fimbriata. OLSON, E. O. Isolations from sweet potatoes affected with black rot, from six States, gave two strains of *Ceratostomella fimbriata*, the typical long-neck perithecial strain and a nonperithecial strain, the latter producing small sclerotium-like bodies. Both strains produced endoconidia, and were pathogenic to sweet potatoes. The two strains were obtained when single ascospores were isolated from the long-neck perithecial strain; this occurred for 4 generations. Single conidial cultures (164) from the long-neck perithecial strain gave only the parent type. When the two strains were paired, a line of perithecia formed where they came together. Ascospores isolated from the line gave the two strains. A third strain, with very short perithecial necks, was isolated also in one case from this line. This short-neck strain formed a line of long-neck perithecia when paired with the nonperithecial strain. Ascospore isolations from this line gave at least three strains, the long-neck, short-neck, and a nonperithecial strain. Ascospore cultures from the short-neck perithecial strain have given rise to the parental type and possibly a nonperithecial strain differing from the original one isolated from the long-neck strain.

A virus causing internal necrosis in White Rose potato. OSWALD, JOHN W. White Rose potato plants with severe leaf, stem, and tuber necrosis were observed in California in 1946. Investigation showed this to be caused by a virus easily transmissible by mechanical means. First generation symptoms consist primarily of internal necrotic browning of veins, petioles, and stems, extreme at the nodes, followed in severe cases by collapse of the entire plant. Affected leaves show irregular necrotic areas and often chlorotic blotches. As the disease progresses these may shrivel and hang. Top leaflets become rugose and tightly curled, and the leaves roll downward. Tuber necrosis occurs first just under the skin giving the surface a purplish cast. Later these areas become sunken and corky and necrosis often spreads through the entire tuber. Second generation internal necrosis is less severe. Potato seedling 41956 reacts similarly to White Rose. Free of the potato latent virus, the necrotic virus induces a chlorotic mottle in pepper, irregular yellow mottle in *Nicotiana rustica*, and green vein banding in *N. glutinosa* and *N. tabacum*. Thermal inactivation is between 50° and 55° C. and longevity *in vitro* between 48 and 60 hours. The virus properties and host reactions indicate a possible relationship to potato virus Y.

Peach X-disease on sour cherry. PALMITER, D. H., AND K. G. PARKER. Both the English Morello and Montmorency varieties of *Prunus cerasus* are affected by a virus that seems to be the same as that causing X-disease of *P. persica* and *P. virginiana*. Symptomatic plants of all three species have been found in close association in several areas of New York. The incidence of diseased sour cherry trees is usually greatest in that part of the orchard adjacent to diseased chokecherries and affected cherry trees have not been found except in association with chokecherries. Symptoms on sour cherry comprise delayed bloom, extensive June drop, especially on English Morello, and the failure of the remaining fruit to mature with full color or flavor. Internodes on the terminals may be shortened, and, during late summer and autumn, some leaves may become abnormally yellow to brick-red along their mid-veins. Graft and bud inoculations, both in the greenhouse and out-of-doors on young cherry trees, caused a wilt often quickly followed by death. Sometimes their leaves became yellow along their mid-veins. Similar symptoms on sour cherry followed the insertion of buds from X-diseased chokecherries. Inoculations from affected sour cherries to peach resulted in typical peach X-disease symptoms. In one orchard 55 per cent of the English Morello trees produced no fruit in 1947 as a result of this disease.

Apple bitter rot on sour cherry in West Virginia. PEET, CLYDE E., AND CARLTON F. TAYLOR. A rot of sour cherry, previously unreported as an economic disease in North America, occurred in appreciable amounts in two orchards and in small amounts in the other orchards examined during the unusually wet 1947 harvest season. The incidence of the rot varied considerably from tree to tree. On some trees 15 per cent (by count) of the fruits were infected, with all fruits attacked on some branches. The rot first appeared as a small tan spot, then progressed rapidly to affect approximately half the fruit area. The surface of the lesion became darker, tended to be folded concentrically, and Gloeosporium-like spores were produced in pinkish masses. Successful cross-inoculation was obtained on Grimes Golden fruits when infected cherries were suspended in cheesecloth bags in the tree a few inches above the apple-or-when an opened

Petri-dish culture of the cherry isolate was suspended similarly. Lesions typical of apple bitter rot resulted. Growth characteristics of isolates from affected cherries were similar to those of isolates from typical apple bitter-rot lesions. Preliminary studies and the successful inoculation of apple suggest that the rot on cherry is caused by *Glomerella cingulata*.

Resistance to powdery mildew in peas. PIERCE, W. H.   In repeated field tests a selection out of the Stratagem variety of garden peas has shown a high degree of resistance to powdery mildew, *Erysiphe polygoni*. Since powdery mildew is often severe in the arid sections of the western States, particularly on late home-garden varieties, a few crosses were made using the resistant Stratagem selection with the standard varieties Glacier and Shasta. In F_2 populations of 330 plants, counts of resistant and susceptible plants gave 243 susceptible and 87 resistant. The total F_2 population (139 plants) of the Stratagem-Shasta cross was tested again in F_3. The plants classified as resistant in F_2 gave F_3 progeny which were also resistant, while plants classified as susceptible in F_2 gave progeny one-third of which were completely susceptible and two-thirds segregating in the approximate ratio of 3 susceptible to 1 resistant. Inheritance appears to be on a single factor basis with resistance recessive. Acceptable horticultural types have been selected from these hybrid progenies and are being increased for introduction.

Types of scab lesions on Camellia. PLAKIDAS, A. G.   Necrotic lesions of various types occur on camellia leaves in Louisiana. The following types are recognized. Type I: General brownish discoloration of the leaf and irregular black spots occurring usually near the margins or irregularly scattered on the upper surface. Type II: Circular to irregular snowy-white, slightly raised, spots on the upper surface. Type III: Raised, brown to blackish, angular spots on the upper surface, 1–20 mm. with prominent angular cracks through the center, and, in the larger spots, with fine concentric cracks. Type IV: Spots on the upper surface, large, typically circular, brown with grayish centers, raised, with very numerous concentric cracks. Type V: Spots typically on the under surface, rarely on the upper. Lesions start as water-soaked pustules, later erupting to form brown, hard, corky excrescences, in small groups, or covering most of the leaf surface. Type VI: Small, circular to irregular, slightly raised, white to light gray, spots on the upper surface. This type occurs most commonly on *C. sasanqua*: the other types are more common on *C. japonica*. The nature of the lesions on camellia was unknown until 1942, when S. C. Arruda isolated *Sphaceloma* sp. from types I, II, and VI. Since then, the writer has repeatedly isolated *Sphaceloma* from lesions of types I, III, IV, and V. It has not been definitely determined if more than one species of *Sphaceloma* is involved.

Cabbage blackleg from seed grown in Puget Sound. POUND, GLENN S., AND O. H. CALVERT.   For more than twenty years the Puget Sound area of western Washington has been this nation's chief producer of cabbage seed, primarily because seed grown there has not been known to carry the blackleg (*Phoma lingam*) and blackrot (*Xanthomonas campestris*) organisms. Such seed has been used commonly without hot-water treatment because of this known freedom from infection, although a mildly pathogenic strain of *P. lingam* has been recorded recently from that area. In 1947 a severe epidemic of blackleg occurred in most cabbage-producing sections of the country. Extensive field surveys indicated transmission from Puget Sound seed. Isolations have been made from several seed lots grown in the Puget Sound area in 1946 and *P. lingam* has been repeatedly isolated from 4 lots, representing a growing area 200 miles in length. Koch's postulates have been repeatedly applied to isolates obtained, establishing proof of blackleg transmission on Puget Sound seed. Certain isolates obtained from seed show cultural and pathogenic characteristics of the mild strain described from the Puget Sound area while the other isolates are typical of virulent eastern strains. This suggests a probable increase in pathogenicity of the Puget Sound strain and renders use of seed grown there without hot-water treatment unreliable.

Strains of cucumber mosaic virus pathogenic on crucifers. POUND, GLENN S., AND J. C. WALKER.   A mosaic disease of the ornamental crucifer, dame's violet (*Hesperis matronalis*), in Wisconsin has been found to be due to two closely related strains of *Cucumber virus 1*. Both viruses were widely pathogenic on members of the Cruciferae including the cabbage tribe. No prominent symptoms, however, were produced on cultivated species tested other than dame's violet. The physical properties of the two viruses agreed very closely with those of *Cucumber virus 1*. The points of inactivation were: tolerance to dilution, 1 to 100,000; aging *in vitro*, 4 to 5 days at 20° C.; thermal inactivation, 70° C. for 10 minutes. One of the two viruses was shown to effectively immunize zinnia (*Zinnia elegans*) plants against Price's no. 6 strain of *Cucumber virus 1*.

Anthracnose of peaches on the market. RAMSEY, G. B., M. A. SMITH, AND B. C. HEIBERG. During July, 1947, Georgia peaches arriving on the northern market showed an unsual type of decay never before observed in commercial shipments. In some car-loads up to 12 per cent of this rot was found in various stages of development. Brown lesions 1/4 to 3/4 inches in diameter were most common. The larger lesions bore acervuli with salmon-pink spores characteristic of the anthracnoses. Numerous isolations from typical lesions always yielded pure cultures of *Glomerella cingulata.* Inoculation experiments showed the organism to be pathogenic to peaches, apples, pears, and plums without evident wounds as well as through wounds. Commercially brushed firm-ripe peaches in contact with mycelium or conidia in a water suspension developed lesions 16 mm. in diameter within a week at 85° F. Under similar conditions inoculations through wounds produced lesions 36 mm. in diameter. No visible infections were obtained within a week when wounded and unwounded peaches were inoculated and held at 45° F.

Metal reagents as antisporulants. RICH, SAUL, AND JAMES G. HORSFALL. The pre-vention of fungus sporulation has been suggested as a possible method of minimizing the loss from phytopathogenic fungi. As fungus spore production has been shown to be related to heavy metal metabolism, compounds which combine readily with metals were tested *in vitro* as antisporulants. The metal-reacting oximes and quinolines were found to be effective in reducing the spore production of *Sclerotinia fructicola, Penicillium* sp., *Alternaria* sp., and *Aspergillus niger.* Dimethylglyoxime, which forms chelate salts with most of the heavy metals, reduced the sporulation of *Aspergillus niger* growing on complete liquid media. Increasing concentrations of dimethylglyoxime decreased sporulation to the point of complete inhibition of spore production without appreciable growth reduction. Yarwood (1941) found that H_2S inhibited the sporulation of *Peron-ospora humuli.* Care was exercised in this study to distinguish between fungistasis and antisporulation.

Pathogenicity of isolates of corn root-rot organisms in Mexico. ROBLES, LEONEL H. Among 146 isolates of the corn root-rot organisms from 8 States, *Helminthosporium sativum* was the most destructive, while species of *Fusarium* were found to be most prev-alent but much less active as root-rot pathogens. In preliminary pathogenicity test four isolates were selected: one of *H. sativum*, two of *Rhizoctonia* spp. and one of *Diplodia zeae.* When these organisms were tested with 26 different types of corn, only *H. sativum* proved to be strongly pathogenic. When all possible combinations among the four isolates were made the mixture of *H. sativum* and *Rhizoctonia* spp. was moderately patho-genic in contrast with *H. sativum* alone. The general result of this combination was a considerable reduction in the percentage of seed germination. -

Barley scald in Mexico. RODRÍGUEZ VALLEJO, JOSÉ. In 1947 barley scald caused by *Rhynchosporium secalis* was reported from Mexico for the first time. The disease was found attacking barley in the experimental plots at the agricultural experiment station in Chapingo, Mexico. Apparently the disease is not widespread and thus far has been found only at the station in Chapingo. Those introduced varieties exhibiting greatest susceptibility are: Club Mariout, Atlas, Velvet, Peatland, Chevron, Kindred, Hanna, Wisconsin Barbless, and Bolivia × Chevron while Mars, Tregal, Manchuria, Bolivia, Svansota, and Texan (CI–5127) were not infected this year. The so-called native barleys planted at the experiment station ranged in reaction from highly susceptible to fairly resistant.

Specificity of fungicides for diseases of bent turf. ROWELL, JOHN B. Five years of investigation have repeatedly demonstrated that no single chemical will completely control all of the following bent turf diseases: dollar spot (*Sclerotinia homeocarpa*), pink patch (*Corticium fuciforme*), copper spot (*Gloeocercospora sorghi*), large brown patch (*Pellicularia filamentosa*) and helminthosporium blight (*Helminthosporium* sp.). Newly developed organic cadmium and mercury compounds have proved to be more effective than fungicides used previously. Cadmium compounds have shown the least specificity; five organic and two inorganic forms control the first two diseases, and three of these organic forms also control copper spot. Five phenyl mercurials compared in 1947 varied in degree of disease control and injury to bent grasses (*Agrostis* spp.) and Crabgrass (*Digitaria sanquinalis*). To date none of the compounds has given significant control of large brown patch or helminthosporium blight. These investigations indicate that a fungicide program may be necessary for maintaining healthy turf.

Root-rot of the Mexican "frijol." RUPERT, J., B. ORTEGA, AND C. CARDONA. During the past three years studies have been in progress with regard to root-rot of the frijol (*Phaseolus vulgaris*), in Mexico. Tremendous variation has been observed within

the varieties commonly grown in Mexico with regard to resistance to this disease, but, with few exceptions, the Mexican bean is susceptible. Two organisms appear to be primarily responsible for root rot of beans in Mexico, and these are *Sclerotium rolfsii* and *Rhizoctonia solani*, although various species of *Fusarium* are frequently isolated and may be involved in this complex. When bean seedlings are inoculated with isolates of *Rhizoctonia* or *Sclerotium*, infection results and typical root-rot symptoms develop. Selections are being made in an attempt to secure resistant lines for a breeding program in 1948.

Development of Cercospora-resistant strains of rice. RYKER, T. C., AND L. E. COWART. To secure varieties of rice resistant to the Cercospora leaf spot (*Cercospora oryzae*) consideration must be given to the parasitism of the different races of the fungus as well as to the resistance of the host. Eight races of the fungus based on their ability to attack 7 test varieties, Blue Rose, Blue Rose 41, Caloro, Fortuna, Red Rice, Rexoro, and Nira have been reported. Two additional races have been identified, race 9, to which Shoemed and three of the test varieties, Blue Rose, Fortuna, and Nira, are susceptible, and race 10, which is similar to race 2 except that it attacks Caloro as well as Blue Rose and Blue Rose 41. Inheritance studies in crosses involving race 6 (Rexoro strain) indicated a single dominant factor for resistance in 5 crosses and two dominant factors for resistance in 3 crosses. All of the commercial varieties used in the rice breeding program are susceptible to one or more parasitic races of the fungus. Delrex, a recently released selection from a cross between Rexoro and Delitus and a number of advanced selections from crosses between Rexoro and Blue Rose are resistant to all of the known parasitic races of the fungus. Thus the factors for resistance in rice to *Cercospora oryzae* may be combined through hybridization.

Chemicals for control of the golden nematode of potatoes. SCHMITT, C. G. Allyl bromide, D-D trimethylene bromide, ethylene bromide, and Iscobrome D were all rather effective in kill of cysts of the golden nematode in a pot test at 23 gal. per acre. Of the chemicals used, the only ones which eradicated under these ideal conditions at 76 gal. per acre were ethylene dibromide and Iscobrome D.

Resistance to apple scab in certain clones of Malus species. SHAY, J. R., AND L. F. HOUGH. Twenty-two clones of *Malus* in plots at Urbana, Illinois, and in Morton Arboretum, Lisle, Illinois, have remained free from foliage and fruit infection by *Venturia inaequalis* during the past three years in which apple scab has been epiphytotic. These clones were grown in the greenhouse where controlled inoculations were conducted. Heavy conidial suspensions from 15 single-ascospore cultures of *V. inaequalis* isolated from 5 apple varieties at Lafayette, Indiana, were combined in each of 4 inoculation series and used singly in 2 inoculation series. On the basis of maximum symptom development, the clones could be divided into four groups: 1. No evidence of infection (*M. toringo* and *M. ioensis*). 2. Numerous or few pin-point depressions with no sporulation (*M. micromalus, M. floribunda*, and 11 other clones). 3. Irregular, necrotic or chlorotic lesions varying from 2 to 5 mm. with no sporulation (*M. baccata jackii, M. sikkimensis*, and a Russian seedling). 4. Lesions few, elongate or irregular, necrotic or chlorotic; sporulation when present sparse (*M. baccata* and 3 other clones). One isolate was unable to produce macroscopic symptoms on *M. sikkimensis*. Heterozygosity and dominance of genes controlling resistance of 8 clones were demonstrated by inoculation tests of seedling, F_1 progenies of these clones crossed with susceptible varieties.

The brown-spot disease of celery. SMITH, M. A., AND G. B. RAMSEY. A serious field and market disease of celery from Colorado not previously reported in the United States has been studied since 1943. The disease is characterized by brown, discolored areas in the form of circular to oblong lesions 1/16 to 3/4 inches in diameter on the inside surfaces of the stalks. On the outer stalk surfaces the lesions are somewhat smaller and more elongated. Infection also occurs on stalklets, in leaflet axils, and on leaflet blades. A *Cephalosporium* sp. constantly associated with infected stalks, stalklets, and leaves on commercial celery bunches has been isolated many times. Typical infections have resulted from pure culture inoculations of celery bunches as well as celery seedlings grown in the greenhouse. The organism has been reisolated in pure culture from lesions resulting from these inoculations. In potato-dextrose agar plate cultures the optimum temperature for growth was 75° F., while growth was not observed at the two extreme temperatures 40° and 90°. Optimum growth of the organism occurred at pH 6.5. There was no growth at pH 4.0.

Classification and identification in Fusarium. SNYDER, W. C., AND H. N. HANSEN. Great variability between clones of wild types, and between their derivatives, is common

throughout the genus *Fusarium*. A classification is required that puts together into one species all such entities of that species, whether they represent clones existing in nature or derived in culture, in spite of any morphologic, biologic or apparent differences resulting from mutation, environment, or host relationships. Such a system would recognize few species, based on morphology, indicate pathogenicity by *forma* names and races within *formae* by numerals, and has been found in practice to facilitate identification. Spore size has been used extensively in the past for speciation, yet spore dimensions of wild types of a fungus vary so greatly as to fall into several of the older species. Consistent differences in spore size do not establish a difference in species because such differences can be shown among single-spore isolates from a given clone. Also, the older concept of basing species on hosts falls apart when required to handle the various races that Armstrong has shown are pathogenic on several different combinations of hosts.

The relation of barberries to physiologic races of Puccinia graminis in the United States in recent years. STAKMAN, E. C., AND W. Q. LOEGERING. With the progress of barberry eradication in the United States there has been a general trend toward stabilization in the prevalence and distribution of races of *Puccinia graminis tritici*. In 1945 and 1946 only four races—17, 19, 38, and 56—were prevalent and widespread in the country as a whole. In the two years, races other than these comprised only 5 and 3 per cent of the isolates identified in areas in which few barberries remain. In barberry-infested areas of Pennsylvania, Virginia, West Virginia, and Washington, on the other hand, new or unusual races or biotypes comprised 26 and 24 per cent of the total number of isolates. The same general relationship appears to hold for 1947. None of the new races or biotypes so far found has a wider host range than race 15B. With one exception, race 7 of *P. graminis avenae*, which attacks White Tartar oats and varieties that derived stem rust resistance from it, has been found only near rusted barberries in Northeastern United States, where it attacked certain normally resistant varieties very heavily in experimental plantings near barberries. Elsewhere, only races 2, 5, 8, and 10 have been found in recent years.

Infection of the sugarcane stalk by red rot. STEIB, R. J. When stalks of sugarcane, which appeared healthy, were surface-sterilized up to 24 hours with a solution of bichloride of mercury and cultured, the red-rot organism (*Physalospora tucumanensis*) was obtained from root bands and buds. It was isolated also from leaf scars and small red areas found in the tissues under the leaf scars. The organism was not obtained from nodes enclosed by tight, healthy leaf sheaths. Several sugarcane varieties developed red rot, even after fungicidal treatments and subsequent storage with the fungicide residue remaining on the cane; the buds and leaf scars served- as the initial points of entry. Various treatments with fungicides and hot water did not eliminate the organism from the stalk. Leaf sheaths of susceptible and resistant varieties collected in the field developed red rot when placed in moist chambers. The perithecial stage occurred on a large percentage of dried leaf sheaths of Co. 290. The evidence indicates that the sugarcane stalk is infected with the red rot organism previous to planting, perhaps in a dormant form, and that the disease develops when or if the vitality of the cane becomes lowered.

Time and frequency of occurrence of Pythium debaryanum and graminicolum on roots of barley. SUMMERS, THOMAS E. Seven field plantings of barley were made from April 2 to July 9. *Pythium debaryanum* was frequently isolated from germinating seeds, roots of young seedlings, and roots of older plants from April 7 through the first week of July. After that time it was isolated with decreasing frequency until July 24 when none was recovered. *P. graminicolum* was not isolated from germinating seeds or from roots of very young seedlings. However, a high percentage of isolations from roots of older plants in all plantings contained *P. graminicolum*. The fungus was recovered frequently from May 2 through August 3. Among isolations made from roots of plants less than eleven days old only 5.6 per cent contained *P. graminicolum*. Among isolations from roots of plants more than eleven days old, 73.5 per cent contained *P. graminicolum*. In no case was *P. graminicolum* isolated from germinating seeds; it was rarely isolated from roots which had not attained a length of 8 cm. *P. debaryanum* was frequently isolated from germinating seeds, roots of very young seedlings and roots of older plants grown in field soil in the greenhouse. *P. graminicolum* was recovered only from roots of older greenhouse-grown plants. Apparently *P. graminicolum* does not infect the germinating seed or roots of very small barley seedlings in field soil.

The effect of cereal crop rotation on "take-all" damage. SUNESON, COIT A., AND JOHN W. OSWALD. A randomized series of wheat, barley, oat, and rye plots grown for hay yield trials in 1946 disclosed severe "take-all" damage in the first two mentioned

crops. The absence of separating alleys and minimum intervening tillage facilitated replanting the same crops in 1947 at right-angles to the previous seeding. From the resulting checkerboard, yields of each crop succeeding itself and each of the other crops, expressed as grams of grain and straw per square foot, were determined from averaging yields at six points. Thus, on areas previously cropped to wheat, the current yields of wheat, barley, oats, and rye were 8, 29, 51 and 55 grams, respectively. On previous barley land these crops yielded 8, 30, 54, and 58 grams; on previous oats, 42, 52, 42, and 51 grams; and on previous rye, 45, 57, 48, and 54 grams, respectively. All isolations from both years' croppings of wheat and barley yielded *Ophiobolus graminis*. No pathogenic fungi could be isolated from oats or rye in either year. The data clearly depict the relative susceptibility of the four crops, their effect on the survival of *O. graminis*, and the benefit from rotating rye or oats with barley or wheat. (Cooperative investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture and California Agricultural Experiment Station.)

Barley powdery mildew (Erysiphe graminis hordei) influenced by environment previous to inoculation. TAPKE, V. F. Wide differences of opinion have long existed in regard to the environmental conditions that promote powdery mildew in barley and wheat. In a preliminary study of environmental influences, two susceptible varieties of spring barley were sown in pots in September, 1946. Soon after seedling emergence some of the pots were put outdoors, some were kept in a greenhouse and lightly watered, and some were kept in a greenhouse and liberally watered. After 8½ weeks the plants grown under these different conditions were mildew-free. They were then uniformly inoculated with powdery mildew conidia and thereafter amply watered and kept in a greenhouse under similar conditions. On the outdoor plants, which were short and heavily tillered, very little mildew developed; on the lightly watered greenhouse plants, a moderate amount developed; and on the heavily watered greenhouse plants, which were tall and succulent, there was very abundant mildew. These results indicate that the pre-inoculation environment may materially affect the post-inoculation reaction of the plants to mildew. This subtle pre-inoculation influence, hitherto unsuspected, apparently, may explain the divergent reports on post-inoculation conditions that promote powdery mildew.

Prolonging viability of spores and mycelium of the barley loose smut, Ustilago nuda. TAPKE, V. F. Spores of many different collections of *U. nuda* and mycelium in seed from hand-inoculated flowers were stored at 32° F. in a refrigerator. At these temperatures some of the spores were still viable after nine years, whereas in storage at room temperatures, viability often declines appreciably in three to six months or less and seldom exceeds one year. Seeds of five barley varieties from flowers inoculated by hand in 1940 and stored at 32° F. were sown in a greenhouse in January, 1947. Sixty-seven per cent of the seeds produced seedlings. In the variety Lion (C.I. 923), 73 per cent of the heads were smutted. Knowledge of the possibilities of cold storage for prolonging viability in *U. nuda* has facilitated study of its physiologic races and breeding for resistance.

Bacteria in healthy plant tissue. TERVET, IAN W., AND JOHN P. HOLLIS. Potato plugs obtained aseptically from many apparently healthy tubers were found to have bacteria associated with their tissues. Plugs prepared with care to avoid superficial or aerial contamination, when placed in tubes of sterile water, not infrequently disintegrated and deposited starch. Besides complete breakdown, 4 additional types of bacterial action were evident: turbidity with gas; green fluorescence; pellicle formation; and partial breakdown without starch liberation. From 15 lots of sound Red Warba potatoes, over 1000 plugs were made and submerged in sterile water. Bacterial development ranged from 10 per cent of the plugs in some lots to about 90 per cent in other lots. Plugs obtained from aerial tubers also were frequently found to contain bacteria. Similarly prepared plugs from storage organs of several plants developed thus: 80 per cent of the turnips gave bacteria, about 30 per cent for carrots, beets, and kohlrabi, while sweet potatoes and tomato fruits remained sterile. Variation in disinfecting procedures and in methods of obtaining plugs did not vary appreciably the percentage of plugs with bacteria. The results indicate that at least some apparently healthy, fleshy storage organs may harbor bacteria.

Germination of spores and artificial culture of Urocystis agropyri from red top. THIRUMALACHAR, M. J., AND J. G. DICKSON. *Urocystis agropyri* occurs on many important pasture grasses. Recently, Fischer suggested the combination of *U. tritici* and *U. occulta* under *U. agropyri*. Germination of the chlamydospores of *U. agropyri* from any of the grass hosts so far is unreported. Chlamydospores on *Agrostis gigantea*

collected at Madison were germinated and the cycle of the fungus studied. A whorl of 2 to 4 sporidia are borne at the apex of the promycelium; these fuse in pairs and develop infection hyphae. Occasionally two or more nuclei pass directly from the promycelium into the sporidia, resulting in infection hyphae without sporidial fusions, as described by Stakman *et al.* in *U. occulta*. Cultures of the fungus isolated have been strictly mycelial types, although cultures from haploid sporidia have not been tested. Chlamydospores developed in culture consisted of spore balls without apparent sterile cells.

The effects of representative plant growth substances upon attenuated-bacterial crown galls. THOMAS, JOHN E., AND A. J. RIKER. Plants inoculated with an attenuated (A6–6) strain of the crown-gall bacteria (*Phytomonas tumefaciens*) have been treated with 57 different, representative plant growth substances. The plants were decapitated and the chemicals in lanoline applied to the cut about 2 inches above the bacterial puncture inoculations. Results were secured from treatments of more than 4900 plants, including tomato, sunflower, Paris daisy, marigold, and velvet leaf. Such plants treated with 19 of these substances developed attenuated-bacterial galls which were larger than those on the controls. These 19 substances represented numerous different kinds of plant growth substances. Attenuated-bacterial-gall stimulation by these chemicals was sometimes, but not always, associated with such growth responses as epinasty, root stimulation, bud suppression, tissue proliferation, or formative effects. Consequently the increased growth of the treated attenuated-bacterial galls may be considered as another type of growth response. Bacteria, reisolated from the stimulated galls were still attenuated. The effect of the growth substances in stimulating the attenuated-bacterial galls appeared to be on the host, or the bacteria-host interaction, rather than on the bacteria.

Differential tests of three isolates of Phytophthora infestans. THOMAS, W. D., JR. One isolate of *Phytophthora infestans* from Maine and 2 from Colorado were tested for their pathogenicity on 10 potato selections and 5 tomato varieties. Of the latter isolates, 1 was from Weld County and 1 from the San Luis Valley. The Weld County isolate was least pathogenic on potatoes, and the Maine isolate was least pathogenic on tomatoes. The Red McClure potato and Pritchard tomato were moderately resistant to foliage infection by all isolates. Simultaneous studies on the retention of sporangial viability in different relative humidities at 24° C. showed that the San Luis Valley isolate survived at a relative humidity of 25 per cent for 3 hours, the Weld County isolate 1 hour at 35 per cent, and the Maine isolate 1 hour at 45 per cent. Some sporangia of the San Luis Valley isolate survived 48 hours in a 55 per cent relative humidity. After 5 hours, 85 per cent relative humidity reduced significantly germination by the Colorado isolates, and 95 per cent reduced the Maine isolate. This evidence indicates that the isolates are separate strains of the pathogen, the Colorado forms capable of surviving conditions of lower atmospheric moisture.

The rôle of minerals in production of streptomycin by Streptomyces griseus. THORNBERRY, H. H. Some plant and animal products that stimulate the production of streptomycin when added to a basal medium (glucose 10 gm., peptone 5 gm., NaCl 5 gm., distilled water 1 liter) were ashed at 700° C. and added to the same medium to ascertain whether the organic or mineral constituents of these products were responsible for streptomycin production. Stimulation expressed as the ratio of production to that in the basal medium (15 units per ml. in 3 days of shaken culture at 26° C.) was obtained: from corn steep 12.9, cowpea seed 12.1, distiller's dry solubles 24.8, peanut meal 9.3, peanut protein 3.4, peanut aqueous extract at pH 8.0 11.5, peanut protein waste liquor 18.5, peptone 6.0, sugar-beet hulls 14.6, sunflower seed meal 23.8, and yeast extract 9.3. There was a reduction in streptomycin production by the ash of some materials: alfalfa meal 0, apple leaves 0.46, chestnut leaves 0.73, and wood sulfite waste 0.93. Peanut meal contained some organic inhibitor since all ashed fractions gave higher production than non-ashed fractions. From this evidence it is concluded that minerals play an important rôle in streptomycin production.

Studies on Xanthomonas pruni bacteriophage. THORNBERRY, H. H., A. EISENSTARK, AND H. W. ANDERSON. *Xanthomonas pruni* bacteriophage has been observed to remain active and specific for its host for 20 years at room temperature, in a sealed glass tube and away from light. Lysis occurred in the first inoculation in which 0.1 ml. of the stored phage was added to a 5-ml. broth culture. The titer of the phage preparation when stored was 10^{-5}. It was not determined when revived. By serial passage through broth cultures with heavy growth (24-hour growth in broth with 1.8 cm.² air surface per ml.), the titer was 10^{-9} on the 12th serial transfer. This preparation of phage lysed all cultures of *X. pruni* that were isolated from peach, plum, and apricot from various parts

of Illinois. It did not lyse *X. lactucae-scariolae*, which is distinguishable from *X. pruni* only by pathogenicity, or *Erwinia amylovora* isolated from apricot twigs, or *X. campestris* var. *armoraciae*. Electron micrographs of the bacteriophage show that it is similar in size and form to *Escherichia coli* bacteriophage and that the bacteriophage is absorbed by *X. pruni*.

Chromates as potato fungicides. THURSTON, H. W., JR., J. G. LEACH, AND J. D. WILSON. Since 1941 numerous compounds containing chromium together with from one to four other metals have been under test, as potato fungicides in Pennsylvania. During 1946 and 1947 certain of the most effective of these have been tested as well in West Virginia, Ohio, and Florida. Certain copper zinc chromates have given outstanding results in the control of both early blight (*Alternaria*) and late blight (*Phytophthora*). When these diseases are serious enough and infection takes place early enough commensurate increases in yield are noted. When used alone, these chromates have been shown to have insect-repellent properties equivalent to Bordeaux mixture and when used with DDT the yields produced have equaled or surpassed the yields from "fixed coppers," "Dithane," and Bordeaux.

An inoculation method for determining resistance in corn to Diplodia ear rot. ULL-STRUP, ARNOLD J. Inoculum was prepared from spores produced on sterile whole oats in 2-qt. fruit jars after 4 to 8 weeks of incubation in the light at 22° to 26° C. Spores from 2 jars of culture were washed free of the substrate and suspended in 1 qt. of water. The final inoculum was made up of 1 qt. of stock spore suspension in 2.5 gal. of water. The inoculum was sprayed over the corn ears by means of an ordinary compressed-air sprayer. Approximately 300 ears could be sprayed with 2.5 gal. of inoculum. The optimum time for inoculation appeared to be a period from full silk until 2 to 3 weeks later. Inoculations made 4 or 5 weeks after full silk resulted in much lower infection. No differential virulence was observed in several isolates tested. Isolates vary considerably in sporulating ability. Selection of isolates was made on the basis of rapid and abundant spore production as determined by preliminary tests. Incidence of infection was determined by (1) percentage of infected ears, (2) by percentage of infected kernels, by weight, in a shelled aliquot of each plot, and (3) by a disease index. Results of the three methods showed a highly significant correlation. Resistance of inbred lines was determined in single-cross combination.

Antibiotics and potato ring rot. VAN SCHAACK, VALERIA. Penicillin and streptomycin kill the potato ring-rot bacterium (*Corynebacterium sepedonicum*) *in vitro*. Therefore, they were applied to artificially inoculated, cut seed pieces. Potatoes used were Minnesota-grown, certified Pontiac and White Rose varieties. Inoculated, but antibiotically untreated seed-pieces of neither variety grew, but rotted in the soil; there was good growth of controls. Inoculated and penicillin-treated seed-pieces did not grow, although controls produced healthy plants. Inoculated, streptomycin-treated seed-pieces gave good growth with both potato varieties.

Combining resistance to wildfire, mosaic, black root rot, and fusarium wilt in burley tobacco. VALLEAU, W. D. In 1946 Ky 23 burley tobacco was pollinated with *Nicotiana longiflora* pollen, a species resistant to wildfire. Several pods were well filled but nearly all seeds were hollow. The forty fertile seeds obtained were treated with colchicine during germination. Of 31 plants, four proved to be fertile when selfed or backcrossed. Resistance to wildfire is dominant, so each generation was backcrossed to eliminate, as quickly as possible, all *N. longiflora* chromosomes except the one, or a portion of it, containing the resistance factor. The backcross parents used were varieties resistant to mosaic (NN) and black root rot; or to mosaic (NN), black root rot, and fusarium wilt. The first and a few of the second backcross generation plants were strap-leaved and rosette-like, but others of the second backcross were excellent type burley plants. The third backcross generation is all tobacco-like. It is hoped that by this method of breeding, varieties will soon be developed that are practically immune to four major tobacco diseases. These varieties of *N. tabacum* will contain genes from *N. longiflora* and *N. glutinosa*.

Factors affecting the nature of resistance of potatoes to scab. VAUGHN, JOHN R. Certain pustule types of scab were first noticed to differ from the common scab type among seedlings tested at the Minnesota Agricultural Experiment Station in 1938. Experiments in the field and greenhouse were conducted for three years at Minnesota and later for one year at West Virginia. These experiments showed that two of the abnormal pustule types selected for study were constantly associated with certain seedling lines, regardless of the race of the pathogen causing infection. This indicated that

the expression of resistance or susceptibility in these cases depended upon the genetic constitution of the host. The Menominee variety, which has consistently been resistant to the attack of many races in different locations in the United States, was investigated to determine the mechanism of its resistance. It was found that the wound periderm formation was completed three times as fast in this variety as in the susceptible Smooth Rural variety. It is suggested that the type of resistance found in Menominee is due to the speed of wound periderm formation, which results in the exclusion of the pathogen from extensive penetration of the host tissue.

Development of clubroot resistance in cabbage. WALKER, J. C., AND R. H. LARSON. Long search for individuals in cabbage varieties, that were resistant to clubroot (*Plasmodiophora brassicae*), has been unsuccessful. In 1941 kale-cabbage hybrid rogues were found in a Wisconsin cabbage field, self progenies of which contained highly resistant individuals. Some of the fourth-generation inbred progenies have uniformly high resistance. These inbreds have the leafy headless kale character. In crosses with cabbage the F_1 generation segregated with a preponderance of susceptible individuals. In the F_2 a small percentage of highly resistant individuals appeared, which were divided about equally between kale, intermediate, and heading plants. From the last group, selections have been made to secure lines in which high resistance is combined with desired head and plant type.

Multiple alleles and factor interaction in Glomerella. WHEELER, H. E., AND S. J. P. CHILTON. Single ascospores from plus-type cultures of *Glomerella* from a variety of host plants invariably produced a high percentage of variants of the minus type. Less frequently variants of other types were produced. During a six-year period a total of eighteen distinct strains, differing in various cultural and morphological characteristics, have been isolated from the progenies of a plus culture from wild morning glory. Analyses of asci produced by appropriate crosses indicated that the differences between seven of these strains were controlled by six genetic factors or groups of factors. Three of these factors were at one locus (A) and three at a second locus (B) on the same chromosome. The factors at each of these loci apparently represent multiple alleles. Two pairs of allelomorphic factors, one at each of the two loci, conditioned the production of fertile perithecia. A factor for partial self-sterility at locus B was epistatic to a factor for self-fertility at locus A when the two were combined in a strain of the minus type. The reverse was true when the same two factors were combined in a strain of the plus type.

Gomphrena globosa, a useful plant for qualitative and quantitative work with potato virus X. WILKINSON, R. E., AND F. M. BLODGETT. Local lesions, without systemic infection, develop when Gomphrena globosa the globe 'amaranth, is inoculated mechanically with potato virus X, provided carborundum is used. Many strains including so-called masked, mottle, and ring-spot strains were tested and all gave this reaction. Lesions appear in 2 to 3 days and are encircled by a red ring within a week after inoculation. Lesions form more slowly and less distinctly on old leaves than on young leaves. Although plants tested from different lots of seed varied considerably in size and appearance, they all appeared to react in the same manner except that some produced many more lesions than others when inoculated with a given inoculum. Since this plant has opposite leaves, which are similar in appearance and sensitivity, it is well-adapted for quantitative work. Most satisfactory results were obtained when plants were used just prior to flowering. At this stage, most plants have 4 pairs of leaves which are large enough for quantitative work. The number of lesions formed is increased if the leaves are rinsed immediately after inoculation, and is decreased if the leaves are shaded for 48 hours before or after inoculation.

A detached leaf method for vector studies with the tomato-spotted-wilt virus. WRIGHT, C. M., AND F. M. BLODGETT. The onion thrips, *Thrips tabaci*, was successfully reared on potato or tomato leaflets placed in Petri dishes containing water. Nymphs were confined on the detached leaflets by a ring of Tanglefoot. Emerging adults were placed in microcages mounted on leaflets. Some adults that had previously fed as nymphs on spotted-wilt-infected potato leaflets produced local lesions when they were confined on healthy detached petunia leaves. These lesions appeared three days after the infective adults were allowed to feed on the petunia leaves, and were similar in appearance to lesions resulting from mechanical inoculation with the virus. The study indicated the importance of maintaining pedigreed cultures of thrips, of testing for the presence of the virus in the leaflets on which the nymphs were to be confined, and of mechanically inoculating one-half of the petunia leaf with the virus to make certain that it reacts with local lesion formation. Leaves of *Nicotiana glutinosa* and *N. tabacum* could not be used to test the infectivity of the adults because the insects failed to feed when confined on them.

Hereditary defects in tomatoes. YOUNG, P. A. Inherited defects in tomatoes resemble viroses or other plant diseases. For example, inherited necrosis caused epinasty of rhachises, browning of veins, yellow spots in leaflets, dying of leaflets, and browning of the epidermis of tomato stems. Inherited lutescence caused yellowing and browning of the lower leaves and yellowing of the stems and unripe fruits. Pits and mosaic-like stripes were inherited in the fruit peel. Brachytic and extremely dwarfed types of growth resembled the bunchy-top viroses. Runty plants remained dwarfed in cold frames. Wiry leaves and midget tomatoes resembled witches' broom or extreme fern leaf. Wilting leaves suggested a root disease. Yellow lethal seedlings died early. Sticky-gene hybrid tomatoes were sterile when they resembled their *Lycopersicum chilense* parent. Inherited susceptibility to physiologic abnormalities occurred in tomato varieties with high percentages of leaf roll, fruit puffing, blossom-end knobs, cuticle cracks, blossom-end rot and catface. A mutant tomato had extremely puffed fruits resembling bell peppers. When genes for resistance to parasitic diseases exist, their alleles for susceptibility are inherited defects, such as susceptibility to wilts caused by *Fusarium* and *Verticillium*, and to leaf spots caused by *Cladosporium* and *Septoria*.

Pod mottle, a virus disease of beans. ZAUMEYER, W. J., AND H. REX THOMAS. A new bean virus, which produces local lesions in certain varieties and systemic infection in others, was isolated in 1945 from mottled bean (*Phaseolus vulgaris*) pods collected in South Carolina. The local lesions caused by this virus are different from those caused by any other virus affecting beans. The systemic mottle symptoms resemble those produced by southern bean mosaic virus but are more intense. Pod mottle virus produces a decided mottling and considerable pod malformation. The virus is readily transmitted mechanically. Immunological studies showed no relationship between pod mottle, southern bean mosaic, and common bean mosaic viruses. Just as in the case of southern bean mosaic, a variety susceptible to local lesion infection is resistant to systemic infection. Similarly, one resistant to local infection is susceptible to systemic infection. Sixty-eight bean varieties were tested and all were susceptible to either local or systemic infection. The varieties susceptible to local infection can be considered commercially resistant. The Sieva Lima bean and soy bean were the only other susceptible hosts found among 31 species representing 20 genera in 9 families. The virus was inactivated between 70° and 75° C. when heated for 10 minutes and at a dilution of 1 -100,000. It resisted aging for 62 days at 18° C., but after 93 it was inactive.

Shiny pod (greasy pod) virus and its identity with black root virus. ZAUMEYER, W. J., AND H. REX THOMAS. A new virus of beans, formerly known as greasy pod and now called shiny pod virus, was described in 1947 as a strain of common bean mosaic virus. It was reported as infectious only to varieties susceptible to common bean mosaic. The identity of shiny pod virus and black root virus has now been established. Varieties such as Idaho Refugee, Logan, and Rival whose resistance to common bean mosaic is inherited from Corbett Refugee develop typical black root symptoms when inoculated with shiny pod virus. In contrast, varieties such as Great Northern 15 and 123 and Red Mexican 3 and 34 whose resistance to common bean mosaic is inherited from Great Northern 1 are resistant. Similarly, Robust does not develop either black root or shiny pod symptoms. Certain pole beans, such as Kentucky Wonder and Blue Lake, develop black root symptoms when inoculated with shiny pod virus. These studies show that black root symptoms develop only on certain varieties resistant to common bean mosaic, and shiny pod symptoms only on those susceptible to it. Although shiny pod virus is widespread in many western bean-growing areas on varieties susceptible to common bean mosaic, black root infection has been found only infrequently on varieties resistant to common bean mosaic, except those deriving their resistance from Great Northern 1.

Pythium seedling rot and root necrosis of Allium cepa. SEMENIUK, G., and B. N. WADLEY. Throughout a growing season *Pythium* spp. attack roots of onion plants grown from seeds, sets, or mature bulbs in peat or mineral soils in Iowa. Diseased roots plated on agar consistently yield high percentages of *Pythium* of spherical and nonspherical sporangial types, including *P. irregulare*, *P. ultimum*, *P. spinosum*, *P. mamillatum*, *P. debaryanum*, *P. graminicolum*. *Fusarium* develops from nearly every infected root, frequently with bacteria and nematodes; less frequent are a chlamydosporous *Phoma* sp., *P. terrestris*, and other fungi. Symptoms of Pythium parasitism are seed rotting, pre-emergence seedling rot, stunted emerged seedlings which often die, seedling damping-off, and lead-colored or faintly brown, yellowish or pink roots lacking cortex in advanced stages of disease. Nearly 100 per cent of pink roots from mineral and peat soils yield *Pythium* spp. with *Fusarium*, occasionally with *Phoma* and other fungi. Isolation techniques for *Phoma terrestris* eliminated *Pythium* spp. Pink root disease of onion in Iowa is primarily a Pythium root necrosis accompanied by *Fusarium* and *Phoma terrestris*.

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FLUORINE ANALYSIS OF ITALIAN PRUNE FOLIAGE AFFECTED BY MARGINAL SCORCH

V. L. MILLER, FOLKE JOHNSON, AND
D. F. ALLMENDINGER[1]

INTRODUCTION

A new pathological condition, herein referred to as marginal leaf scorch, was reported in Italian prune trees in several localities in western Washington during the middle to latter part of the growing season following production of aluminum in those areas (Fig. 1). The injury was investigated in
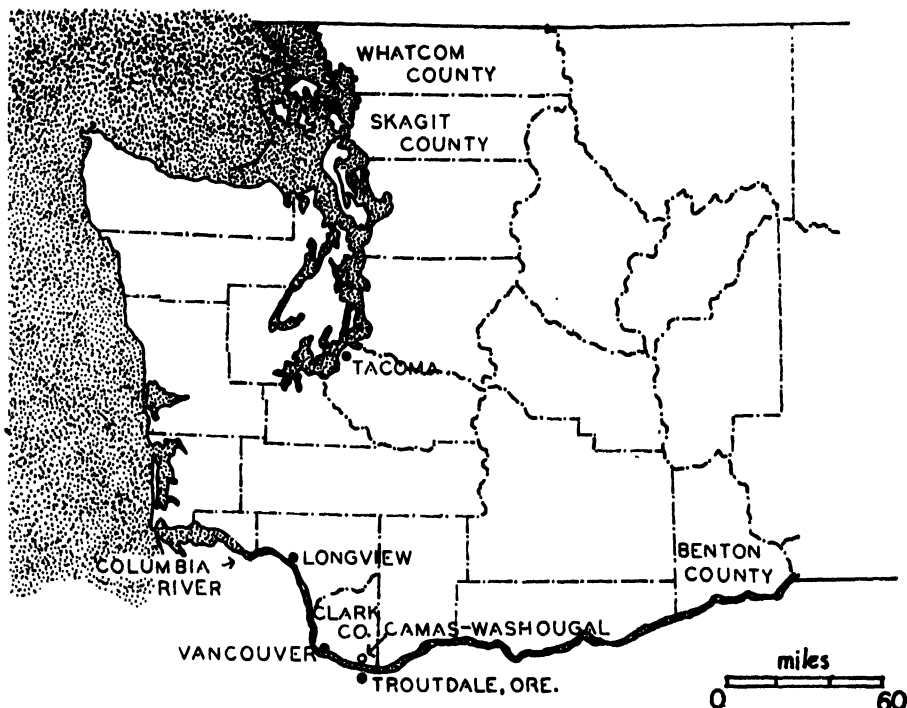
FIG. 1. Part of Washington showing locations where samples of prune leaves were collected for fluorine analyses. Solid circles indicate location of aluminum factories.

1944, 1945, and 1946. It was found to occur in the areas of Washougal, Vancouver, Longview, and Tacoma in 1944 and 1945. In 1946 the leaf scorch occurred in the areas of Vancouver and Longview, but not near Washougal and Tacoma, following closure of the aluminum factories in the latter places. Fluorosis in cattle had been diagnosed in the area of each aluminum factory in Washington and the one at Troutdale, Oregon.

All prune trees in an orchard usually within an area of 10 miles or less, but varying considerably with each factory in question, may be affected to some degree. Neither soil type nor cultivation greatly altered the condition, although the symptoms decreased with abundant water supply. All attempts to isolate an organism as a causative agent failed, and examination of indicator plants eliminated sulfur dioxide as a causative agent.

SYMPTOMS

The first symptoms on Italian prune trees appear as water-soaked areas around the leaf margin, or less frequently they may appear in the leaf lamina. The affected tissue darkens to a brown and may fall out (Fig. 2).
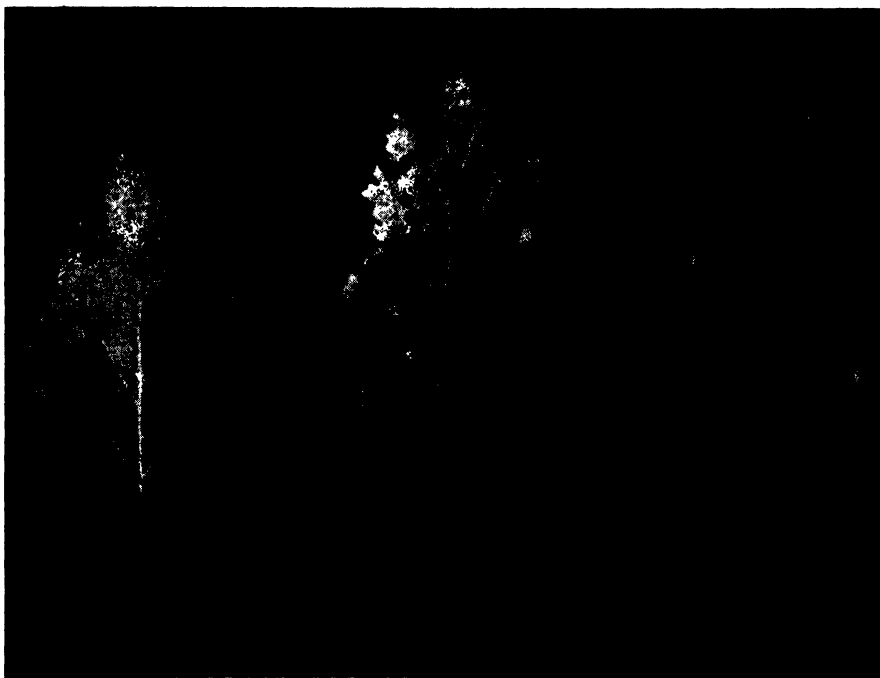
FIG. 2.   Italian prune leaves showing different amounts of injury.   Leaf at left shows marginal scorch only; two leaves at right have symptoms of marginal scorch and leaf spot.   Samples collected near Washougal, Clark County, Washington.

All leaves may not be affected, but usually the lower half of the tree is more severely injured than the upper part. Where leaf scorch is severe as much as 50 per cent of the leaves, especially from the lower branches, may fall by the first part of August. In 1943 in the Washougal area the injured trees dropped most of the fruit two to three weeks before harvest and failed to set fruit in 1944 and 1945. A somewhat similar leaf condition in an apricot and fig orchard near an aluminum factory in California was reported to be caused by fluorine (8).

The production of leaf scorch in Italian prunes and other plants by fumigation with hydrogen fluoride in a sealed chamber has been reported by the

authors (16). A detailed report of this part of the work will be the subject of a subsequent paper.

Fluorine compounds may occur in the smoke from industrial plants using fluorides in their processes. Injury to vegetation by fluorine compounds has been reported in the neighborhood of aluminum, beryllium, copper, iron, ceramic, glass, brick, superphosphate, and miscellaneous chemical factories (3, 4, 5, 13, 14, 15, 23 through 27, 29, 30). The fluorine-containing compounds have been reported to be hydrogen fluoride (HF), hydrofluosilicic acid (H_2SiF_6), and silicon tetrafluoride (SiF_4) (5, 13, 14). These compounds, when present in the atmosphere even in very minute concentration, have the property of being absorbed by moisture droplets, such as dew or fog, to increase very greatly the concentration in a limited area (2, 14, 18, 26, 27, 29). In solution in dew hydrogen fluoride acts as a caustic acid. When the dew evaporates gaseous hydrogen fluoride may enter the leaf as a caustic gas (13). Thus the poisonous action of hydrogen fluoride, as both acid and gas, may be accomplished although the atmospheric concentration is .very low. In a closed room injury to vegetation was observed following a three- to four-week exposure when the atmosphere contained as little as two p. p. m. of hydrogen fluoride (13). Under somewhat different conditions two p. p. m. of silicon tetrafluoride caused injury much more quickly (30), as did higher concentrations of either material (13, 30).

Descriptions have been given of the symptoms of injury from fluorine-containing gases for a number of species of trees, together with detailed microscopic study of changes in leaf structure (5, 13, 14). The description of the macroscopic injury to prune leaves is similar to that observed in western Washington. However, several investigators have pointed out that diagnosis of fluorine injury to plants cannot be proved by symptoms alone, since other factors such as climatic conditions and other industrial fumes may cause similar symptoms (5, 13, 14, 15, 17, 24). Chemical analysis of the injured tissue was used as the final proof of fluorine injury by these investigators.

The normal fluorine content of air-dried plant material, exclusive of roots, grown under normal conditions recently has been reported to be from 0.2 to 4.5 p. p. m.; fertilization of the soil with fertilizers containing fluorine has a negligible effect on the fluorine content of the. foliage (6, 12, 13, 19, 20, 21, 22, 26, 28). However, a report from French Morocco (10) indicates that where ground-water fluorine is high, the fluorine content of some plants may be higher. Earlier reports (11) that dried plants normally contain 130 p. p. m. or more of fluorine are believed to be much too high, possibly due to inaccurate chemical methods available at that time for the determination of fluorine (5, 7).

Considerable variation has been reported in the susceptibility of different plants to fluorine compounds in the atmosphere. Hydrangea and tomato were reported more resistant than *Asparagus sprengeri* Regel., *Cyclamen sp.*,

and *Solanum capsicastrum* Link. (9). Ash has been reported to be more susceptible than several species of oak, maple, and beech (6) ; while conifers, such as pine and fir, are very easily injured (6, 14, 30). Very sensitive to hydrogen fluoride fumigation of 10 p. p. m. were willow, birch, sweet cherry, nut, plum or prune trees (Pflaumenbaum), potato and grape vines. More resistant were sour cherry and apple trees. The pear tree was very insensitive, and almost as resistant were locust, alder, oak, poplar, and elm (14). Under field conditions it was reported that beech, oak, linden, chestnut,

TABLE 1.—*Fluorine content of prune leaves collected in 1944, 1945, and 1946 from several areas in Washington where aluminum factories were in operation at time of sampling*

Location of sample (area)	Location of nearest aluminum factory	Distance from nearest aluminum factory		Sample date (approximate)	P. p. m. F in dry matter			Number of samples analyzed
		Miles	Direction		From	To	Av.	
Vancouver	Vancouver	0–1		a	137	199	168	2
		2–5	N to E	b	21	62	39	14
		5–10	N to E	b	13	80	30	11
		over 10	N to E	b	10	25	19	11
Portland (Ore.)c	Vancouver	3–6	S	b	88	132	110	2
Washougal	Troutdale (Ore.)	2	NE	Oct. 15	231			1
		2–5	N to NW	b	30	37	34	2
		2–5	NE to E	b	83	161	121	7
		5–10	NW	Sept. 1	21	31	26	2
		over 10	NW	Sept. 1	18			1
Longview	Longview	0–3	W to NE	Oct. 15	61	65	63	2
		0–3	E to SE	b	110	420	274	13
Tacoma	Tacoma	0–3	N to SE	Nov. 1	130	1400	496	8
Whatcom and Skagit Counties	Tacoma	over 90	N	Oct. 15	6	15	9	5
Benton County	Troutdale (Ore.)	over 100	E	Oct. 1	8			1

a Samples taken approximately Oct. 15 and June 15.
b Samples taken at various times after July 15.
c Obtained from J. S. Wieman, Oregon State Department of Agriculture, Portland, Oregon.

walnut, grape, Damson plum, Myrobalan and Yellow Egg plum showed injury from fluorine-containing gases, while locust, maple, and blackberry showed only a trace of injury and apple and cherry were uninjured (14). The storage of fluorine in the young bark of trees exposed to fumes containing fluoride has been reported, but the translocation of fluorine into the new leaf growth did not occur (6). Thus scorch on new young leaves cannot be caused by stored fluorine (6).

MATERIAL AND METHODS

Leaf samples showing injury were collected from several localities in Clark County, Washington, where disease symptoms were acute, and from localities where the characteristic injury was slight or absent. In addition,

TABLE 2.—*The effect of operation of aluminum factories on fluorine content of Italian prune leaves*

Location of sample (area)	Location of nearest aluminum factory	Distance from nearest aluminum factory (miles)	Av. p. p. m. F in dry matter		Number of samples	
			1945	1946		
			Aluminum factory		1945	1946
			Operating	Not operating		
Washougal	Troutdale (Ore.)	2–5	121a	9b	7	5
Tacoma	Tacoma	0–3	496 (Nov. 1)	20 (Oct. 7)	8	2
Vancouver	Vancouver	2–5	Operating 35a	Operating 45b	8	6
Longview	Longview	0–3	303 (Oct.–Nov.)	179 (July 25)	10	3

a Samples taken at various times after July 15.
b Samples taken at various times after June 15.

prune leaf samples were also collected in the vicinities of Longview and Tacoma where leaf scorch was also prevalent. To determine the fluorine content of prune leaves in a disease-free area, leaf samples were collected in the northwestern and central parts of the State, ninety miles or more from the nearest aluminum factory. In all cases the samples were dried in an oven at about 75° C., ground in a Wiley mill, and analyzed for fluorine according to the method recommended by the Association of Official Agricultural Chemists (1, 7). Samples of twigs taken during the dormant season were divided into the preceding season's growth and earlier growth. They were dried, ground, and analyzed by the same procedure as that used for the leaves.

CHEMICAL ANALYSES

The results of the chemical analyses of leaves are shown in table 1. The data recorded show that in areas near the aluminum factories the fluorine content of the leaves was higher than in distant areas. This was especially

TABLE 3.—*Fluorine content of prune twigs of different ages*

Location of sample	Distance from nearest aluminum factory (miles)	Age of twigs and Av. p. p. m. F		Total variation	No. samples for each age
		1 year	2–3 years		
Clark County	2.5 –10	2.2	2.6	1.3–5.0	3
Tacoma	0.25	17.0	16.0	....	1
	0.50	9.3	6.6	....	1
	0.5 – 3.0	4.1	3.5	1.9–4.7	3
Whatcom County	100	0.7	1.0	0.5–1.1	2

true to the east and south of the factories but considerable variation occurred within an area.

The results in table 2 show the effect of the operation of aluminum factories on the fluorine content of Italian prune leaves. The fluorine content of prune leaves in the area of closed factories returned to approximately normal.

In order to determine if fluorine is stored in the tree, samples of twigs were taken in the dormant season and analyzed. The results of these analyses are shown in table 3. Although some fluorine was stored in the twigs and branches of injured trees, there was up to 100 times as much fluorine in the leaves.

<div align="center">DISCUSSION</div>

In the Washougal and Vancouver areas where the summer rainfall is light, the severity of the injury was accentuated. There definite leaf scorch appeared with as little as 25–30 p. p. m. of fluorine in the leaves. It was observed in one orchard that trees watered by a sprinkler showed less injury than adjacent non-irrigated trees, although leaf samples from both trees contained 83 p. p. m. of fluorine.

In 1945 prune leaves from the area near Vancouver generally contained less fluorine than those in the vicinity of Camas and Washougal. The injury around the latter places fanned out in a wide area north of the factory at Troutdale, Oregon. That year the fluorine content of the leaves was higher northeast and east of the factory than was recorded at comparable distances to the northwest. The pattern of the injury around Vancouver was more general. All samples from the Longview area were taken close to the factory in question and were relatively high in fluorine and showed considerable marginal scorch. The same was true of the Tacoma area in 1945. In contrast, however, in 1946 when the factories were closed, the leaves did not show marginal scorch and contained 25 p. p. m. or less of fluorine. The same was true in the vicinity of Washougal. These results in general confirm the observation of previous investigators that fluorine is not translocated to new leaf tissue (6). In the areas around Vancouver and Longview, the amount of fluorine in the leaves and the degree of injury was similar in 1945 and 1946.

The samples taken from Whatcom, Skagit, and Benton Counties, which are over 90 miles from aluminum factories, may be considered to contain approximately the normal amount of fluorine for factory-free areas in Washington.

<div align="center">SUMMARY</div>

A new non-parasitic disease of Italian prune trees characterized by marginal scorch and leaf spot appeared in certain areas of western Washington. In an investigation of the cause of the condition, it was shown by chemical analysis that leaves from trees grown in the affected areas were abnormally high in fluorine, as compared with leaf samples collected from other locali-

ties where this condition does not occur. The analyses from six samples collected 90 miles or more from an aluminum factory ranged in fluorine content from 6 to 15 p. p. m., while 76 samples from areas within 20 miles of an aluminum factory varied between 30 and 1400 p. p. m. of fluorine.

Within a given area the amount of marginal scorch was approximately proportional to the fluorine content of the leaves. The fluorine content of the prune leaves returned to near normal and marginal scorch of the leaves did not appear during the growing season following the closure of the two aluminum factories in Tacoma and Troutdale, Oregon.

The evidence indicates that some fluorine is stored in dormant twigs, but is not translocated to any great extent into the growing leaves.

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SOIL FUMIGATION FOR FUNGUS CONTROL WITH
METHYL BROMIDE

A. G. Newhall and Bert Lear

In the search for volatile fumigants that can be used to control not just nematodes but damping-off fungi, root and stem rots, and sclerotia of *Sclerotinia*, the writers' experience with methyl bromide seems worth recording at this time. The results of five tests indicate that the ease with which methyl bromide can be handled, the absence of lachrymating and of dangerous phytocidal properties and its rapid penetrating power recommend it to some florists and vegetable plant growers who want a quick, labor-saving way to treat their seed bed or potting soil and are willing to pay a premium for a less disagreeable and quicker method than formaldehyde, steam, or chloropicrin can offer.

Methyl bromide has been employed in several ways in different experiments. The pure liquid, which comes in pound cans and cylinders under pressure at room temperatures, has been liberated in soil placed in 55-gallon, covered, metal drums. In a similar manner one- and two-cubic-yard piles of soil have been treated under a gas-proof tarpaulin. Solutions of methyl bromide on the market as Dowfume G and Iscobrome have also been used by injection methods at one-foot intervals in low piles or lesser intervals in flats of soil and immediately sealed in. One grower has been applying one of the above products by a sprinkler technique, at intervals over the soil, while building his compost pile with a steam shovel.

In 1945 comparative tests made by the junior author with several fumigants in gallon crocks of soil infested with species of *Pythium* and *Fusarium* showed that as the dosage rates of Dowfume G and Iscobrome were increased well above those needed for nematode control, the control of damping-off of peas was improved. With this and other clues as to the possible efficacy of methyl bromide as a soil fungicide, two 55-gallon metal drums were filled with a compost soil obtained from a florist in Rochester. The soil in one had a temperature of 48° F. and in the other 60° F. at the time of treatment. The drums had rubber gaskets and tight-fitting lids. Soil moisture was adequate for good seed germination. Exactly 90 ml. of methyl bromide was metered into the center of each drumful of soil through "Saran" tubing inserted through a rubber stopper.[1] Since there were 8 cu. ft. of soil in each drum, the amount of methyl bromide used was approximately 11 ml. per cu. ft. The drums were held in rooms at approximately 48° F. and 60° F., respectively, for 6 days after which samples of soil both treated and untreated were placed in flats and sown with spinach and beet seeds. The results are given in table 1. The grower used the balance of

[1] A convenient metering device for methyl bromide is made by the Arrow Products Company, Carlstadt, N. J.

TABLE 1.—*Results of soil treatments in metal drums*

Treatment	Average Percentage germination		Number of weeds
	Spinach	Beets	
Check	2	16	27
Methyl bromide 90 cc.—48° F.	62	88	4
Methyl bromide 90 cc.—60° F.	45	93	0
Steamed 30 min. at 15 lb. pressure ...	50	100	0

the soil to raise snapdragon seedlings. These are very subject to damping-off but for the first time in years he had practically no loss and almost no weeding to do.

In August, 1945 Stark and Lear treated two compost piles comparing a 10 ml. per cubic ft. rate of chloropicrin (Larvacide) with a 50 ml. rate of Dowfume G. The soil temperature was 75° F. Injections were made 3 inches deep and the piles sprinkled with water after which the chloropicrin-treated pile was covered with wet canvas and the Dowfume-treated one

TABLE 2.—*Results of compost pile treatment with Larvacide and Dowfume G*

Treatment	No. of weeds	Stands	
		Cucumbers Per cent	Spinach Per cent
Check	1287	54	16
Larvacide at 10 ml./cu. ft. Pile sprinkled and covered with wet canvas ..	14	87	64
Dowfume G at 50 ml./cu. ft. Sprinkled and covered with gas-proof tarpaulin	3	80	76

with a gas-proof tarpaulin. At the end of 5 days, samples of treated and untreated soil were sown to spinach and cucumbers. The final stands of seedlings and of weeds are given in table 2 from which it can be seen that under these conditions, methyl bromide performed as well as chloropicrin both as fungicide and herbicide.

The importance of adequate confinement of the fumigant was indicated by a similar experiment in September where liquid methyl bromide was

TABLE 3.—*Results of compost pile treatments with methyl bromide*

Methyl bromide treatment	No. of weeds per flat	Average percentage stands (3 flats)		
		Spinach	Cucumber	Sweet pea
Check—None .	114	20	25	20
½ lb. per cu. yd. (under gas-proof tarpaulin)	0	18	51	29
1 lb. per cu. yd. (under gas-proof tarpaulin)	0	43	80	60
1 lb. per cu. yd. (under canvas cover, not gas-proof)	21	10	20	27

discharged from a one pound can under different coverings into 1 and 2 cu. yd. piles of compost soil at Auburn, N. Y. Soil temperature was only 56° F. The results, as measured by seedling growth in samples of the soil, are given in table 3, and are shown in figure 1.

Again 12 ml. methyl bromide per cu. ft. was adequate at the rather low temperature of 56° F. There is reason to believe from the work of the U. S. Bureau of Entomology that at soil temperatures about 20° F. higher a 25 to 50 per cent reduction of this dosage might be adequate under proper conditions of confinement.

On February 11, 1947, a quantity of sandy loam compost heavily infested with weed seeds and damping-off fungi was brought indoors from cold frames and treated in a layer 1 foot thick on a concrete floor in a farm building near Rochester, N. Y. The soil moisture was fairly high but temperature was only 52° F. Injections of 60 ml. of Dowfume G were made on 12-inch centers about 5 inches deep. A gas-proof tarpaulin was

TABLE 4.—*Compost pile injections with Dowfume G*

Crop sown	Mean percentage stand of four replicates		Total no. weeds (2 flats)	
	Untreated	Treated	Untreated	Treated
Beets	60	95	225	72
Cucumber	46	83	237	104
Peas	7	94	462	176[a]
Tomatoes	42	66		
Spinach	10	32		
	165	370		

[a] All weeds in treated flats turned out to be clover while purslane and grasses were also present in checks.

used as cover and left on 48 hours. Samples of treated and untreated soil were brought to Ithaca and sown with 5 different vegetables. Results of this test are given in table 4, and may be seen in figure 2.

The grower made a similar test of treated and untreated soil using lettuce as an indicator, results of which were equally striking (Fig. 2, B), namely a 50 per cent increase in stand with a corresponding decrease in weeds due to treatment. The same grower, the year before, had treated part of his soil in the metal drums using Iscobrome, and noted much better growth of his melon seedlings.

In another test,[2] in which Dowfume G was compared with steam, formaldehyde, and chloropicrin, soil was treated *in situ* in flats ($14 \times 18 \times 3$ inches) with the methyl bromide fumigant by pouring 5 ml. in each of 4 spaced holes ($1\frac{1}{2}$ inches deep). The treated soil was immediately sealed by sprinkling lightly with water, then the flats were covered with glue-coated paper and stacked in piles for two days at approximately 75° F. In other stacks were steamed, chloropicrin-treated, and formaldehyde-treated flats. The stands of seedlings are given in table 5.

[2] Test made by students in plant disease control course taught by Dr. L. J. Tyler.
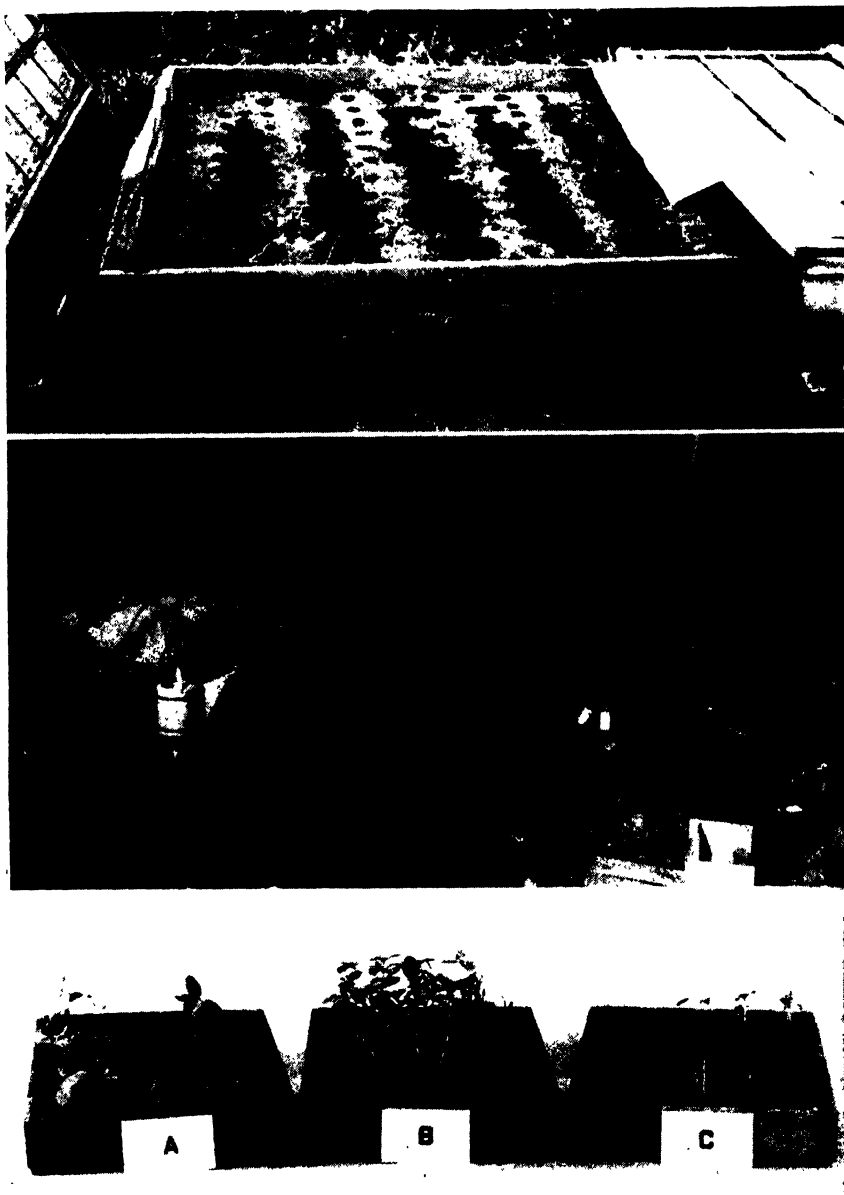
FIG. 1. *Top:* Coldframe treatment with straight methyl bromide showing holes punched in soil at 6- to 8-inch intervals, method of covering with glue-coated paper, and three strings attached to buried sclerotia. *Center:* One- and two-cubic-yard piles of compost soil being treated with one-pound cans of methyl bromide under different types of cover. Strings are attached to bags of buried sclerotia. *Bottom:* Some results of treatments given above. A, Check. B, one pound methyl bromide per cubic yard under gasproof tarpaulin. C, same under ordinary water-proof tarpaulin. (Mere water seal was also inadequate.)
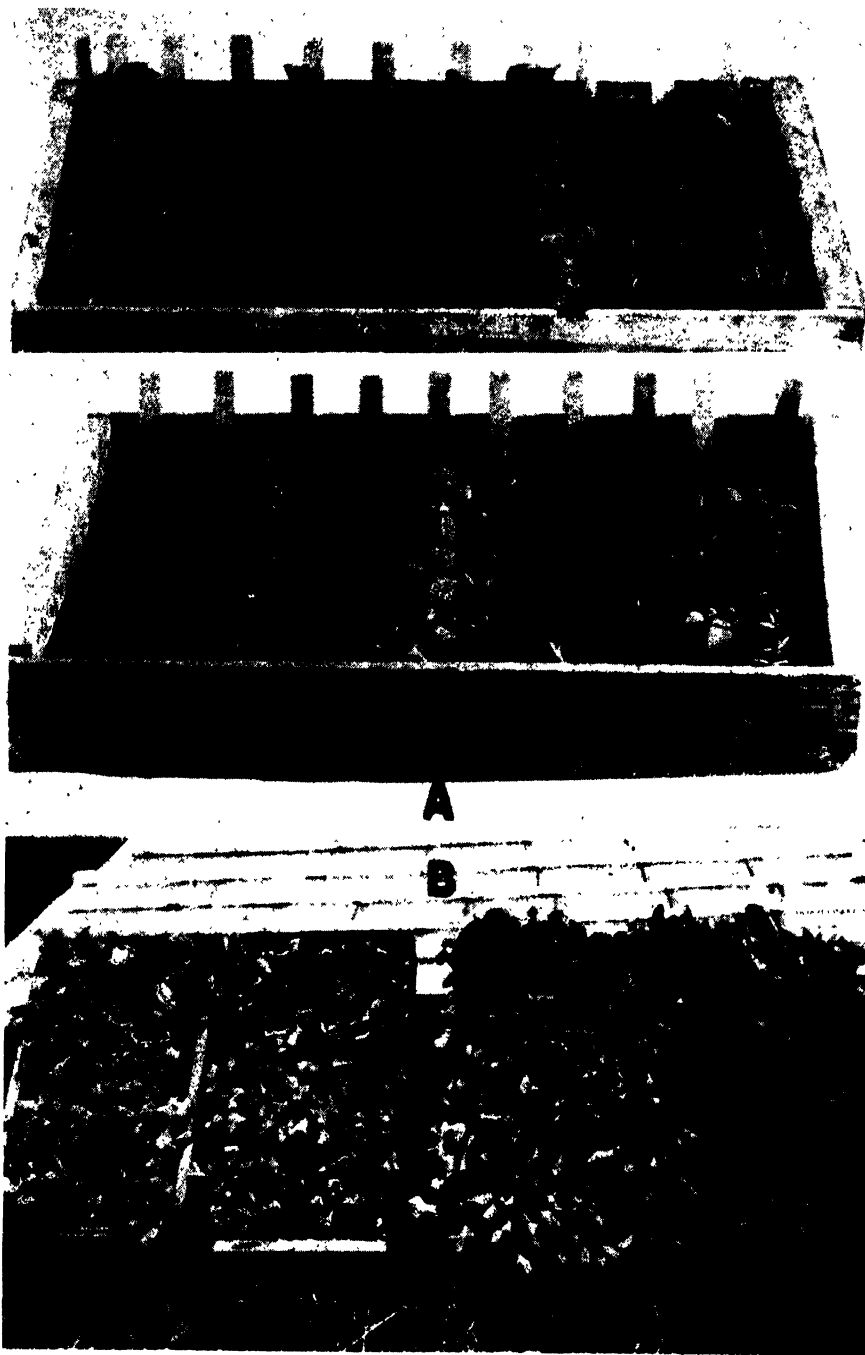
FIG. 2. Results of cold-frame soil treatment with Dowfume G at 60 cu. cm. injections on 12-inch centers covered 2 days with gas-proof tarpaulin. A. Upper flat treated, lower check. B. Two flats on left untreated soil, right treated.

At the rate employed, a pound of Dowfume G at 35¢ would treat 16 flats, at a cost for material slightly over 2¢ a flat. It is probable that several layers of wet newspaper could be successfully substituted for glue-coated paper as a wrap for the flats.

On three or four occasions the sclerotia of *Sclerotinia sclerotiorum* have been buried in soil which was being treated with either straight methyl bromide at 11 ml. per cu. ft. or with Dowfume G at 50 ml. per cu. ft. Even at soil temperatures between 50° and 55° F., complete kill has been obtained provided the sclerotia were soaked a few hours prior to fumigation. When they were dry, many of them survived the treatment regardless of soil temperature.

These methods of treating soils with either straight methyl bromide or with commercial preparations containing this fumigant will be found useful in situations where a grower wishes to obtain fungus control and is willing to pay a small premium in order to avoid the disagreeable, or the

TABLE 5.—*Results of treating flats of soil*

Crop sown	Final stands of seedlings (2 flats)				
	Check	Steam	Chloropicrin	Formaldehyde	Dowfume G
Cucumbers	5	28	25	33	32
Spinach	5	29	49	36	35
Tomatoes	16	17	19	23	31
Beets	25	115	98	117	106
	51	189	191	209	204

phytocidal drawbacks of chloropicrin fumigation or the labor and time required in the formaldehyde treatment of small quantities of soil. One advantage of using straight methyl bromide in cases where the soil can be held in a gas-proof drum or other container is the short time required for the entire operation. It is believed that a 12-hour treatment followed by a similar period of aeration will be found ample time to keep soil out of use, wherever seeds are to be sown. Further experiments may show that results can be obtained with lower dosage rates than the ones recorded here.

While methyl bromide so far has been tested against only damping-off fungi and the sclerotia of *Sclerotinia sclerotiorum*, it seems entirely possible the methods outlined may be effective in eradicating other pathogens. The potency of this fumigant against nematodes, when used in metal drums was pointed out by Taylor (1941)[3] who found 1 ml. per cu. yd. adequate. Since methyl bromide fumes are practically odorless and dangerous to man, the operator should always provide plently of ventilation when working around or with it.

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CLITOCYBE ROOT ROT OF CITRUS TREES IN FLORIDA

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INTRODUCTION

Numerous cases of mushroom root rot, caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres., have been recorded by the writer since 1924 on a large variety of woody plants from various parts of Florida, where the disease is known to have occurred as early as 1902 (14). In addition to attacking native forest trees, this fungus attacks tungoil trees and a large number of fruit trees and ornamental trees, shrubs and vines, including many exotic ones. It was not until the end of 1929, however, that definite evidence was secured that citrus trees also are attacked. At that time, specimens of the roots of rough lemon (*Citrus limon* (L.) Burm. f.) under grapefruit at Lake Alfred, Polk County, sent to the writer for diagnosis, were found attacked by root rot, and pure cultures of *C. tabescens* were obtained from them. An inspection of this grove early in 1930 revealed that this disease was extensive in the root systems of a considerable number of 15-year-old grapefruit trees. During this and succeeding years root rot was found in other citrus groves at Lake Alfred and in groves at a number of other points in Polk County, as well as in other counties in Florida. Preliminary reports of its occurrence have been presented by the writer(7 to 21).

Although the occurrence of Clitocybe root rot of citrus trees in Florida was not recognized until late in 1929, this disease undoubtedly has caused considerable loss of trees in groves for many years. One grower informed the writer that this was the disease for which he began treating in 1915 in his grove at Kathleen, Polk County, where it was still attacking trees in 1932. A further record of what clearly was this disease is afforded by notes, dated August, 1916, found several years ago in the files of the Florida Agricultural Experiment Station. These were based on a letter and specimens of the butt of a rough lemon under grapefruit sent by Mr. S. F. Poole of Lake Alfred. The writer has found Clitocybe root rot prevalent and destructive in a number of the groves at Lake Alfred formerly in Mr. Poole's charge. It is believed that both records constitute definite evidence that this disease occurred in the citrus groves of Polk County at least 15 years before its presence was demonstrated.

DISTRIBUTION AND ECONOMIC IMPORTANCE

No opportunity has been afforded to make a systematic survey of the citrus region of Florida to determine the exact distribution of Clitocybe root rot, though a number of groves at various points in Polk and Brevard

Counties, where this disease was reported or suspected to occur, have been examined in more or less detail for 10 years, beginning in 1930. Observations in other counties have been largely incidental to other projects.

Unfortunately, even in groves where this disease occurs, unless fruiting bodies of the causal fungus develop, it is difficult to locate attacked trees without digging to expose the root systems for examination. In the absence of the fruiting bodies, attacked trees rarely have symptoms of the disease until the root systems have been killed to such an extent that the trees begin to decline, and such symptoms may be indicative of a number of troubles.

Clitocybe root rot of citrus trees has been found thus far principally in various sections of Polk County, where plantings are almost entirely on rough lemon stock. This disease also has been found to a limited extent in a few groves in Brevard County, where this rootstock is used but rarely. It also has been found occasionally in Lake, Marion, and Highlands Counties.

Clitocybe root rot has been found more or less frequently in Polk County in several groves in the vicinity of Lake Alfred, in a few around Winter Haven, in 3 in the Lakeland Highlands section, 2 near Waverly, 2 at Lake Hamilton, 1 at Kathleen, 1 near the south end of Lake Juliana 7 miles north of Auburndale, and 1 at Lake Wales. The prevalence of this disease at several more or less widely scattered localities in this county indicates that a more extensive survey will establish its occurrence at other points.

In Polk County, where Clitocybe root rot of citrus trees is most frequent and widespread, the incidence of the disease, where found, varied within wide limits. The first examination usually did not suffice to locate all the attacked trees in most of these groves, and in some from one to several additional ones were found at subsequent inspections. In some cases the attacked trees occurred in groups but more often they were scattered irregularly throughout the groves or parts of them, indicating various centers of infection.

At Lake Alfred the number of trees attacked varied from one or a few in 5 groves to at least 15 or 20 in another, and 40 in still another. A survey of 6 of these groves under one management,[2] supplemented by subsequent inspections in some, resulted in locating a total of 80 attacked trees, comprising 0.2 per cent of approximately 40,000 trees on about 700 acres. Since these groves average about 60 trees per acre, the total number of attacked trees would equal about 1⅓ acres out of the 700. These groves were from about 12 to 19 years old.

In the groves examined in the vicinity of Winter Haven the incidence of the disease mostly ranged from but a single tree to a few scattered ones, though in one grove visited later 30 trees about 15 years old were being

given surgical treatment after the disease had progressed so far that they had commenced to decline.

In the first grove inspected for root rot in 1931 at Highland City in the Lakeland Highlands section, where a considerable number of trees were reported to be dying, it was found that 34 out of a total of 384 on the first 16 rows inspected were attacked and had been given surgical treatment. Forty-eight trees in this 10-acre block had been removed on account of decline from root rot. An examination of the diseased roots from the 34 trees treated a short time previously showed that the trouble was due to Clitocybe root rot. In another 10-acre block, 14 years old, later examined in this section the owner stated that 8 per cent of the trees were attacked by this disease and that 28 trees had been given surgical treatment a short time prior to the inspection. This disease was found to occur frequently in another grove in this section that was examined by superficial digging around suspected trees, and growers have reported its occurrence subsequently in others.

Clitocybe root rot also occurred extensively in portions of a grove at Kathleen, and the former owner[3] reported that losses began about 1915, when the trees were about 5 years old.

A large grapefruit and an orange, both on rough lemon stock, were found attacked by Clitocybe root in a block of grove at the south end of Lake Juliana 7 miles north of Auburndale, in which several old seedling sweet orange trees having a number of dead and rotted roots had been treated surgically several years before for a disease thought at the time to have been foot rot (*Phytophthora parasitica*). Further evidence of the activity of Clitocybe root rot in this property was afforded by finding it causing the death of several trees in a commercial planting of guavas (*Psidium guajava*).

In Brevard County, the only other county in which Clitocybe root rot was found occurring to any particular extent, this disease was limited to a few groves in the central portion. It was most frequent in portions of two groves on rough lemon stock at Georgiana on Merritt Island. In one of these, where the trees were 15 years old, 38 trees in one corner were attacked. In the other, where the trees were 10 years old, 10 scattered trees were attacked. Instances of the disease on single trees on the same rootstock were found at Courtenay on Merritt Island, City Point, and Melbourne. The one at Melbourne was a calamondin in the yard of a residential property where a number of ornamentals also had died from the disease. This

disease also was found to have attacked extensively the root systems of four seedling sweet orange (*Citrus sinensis* (L.) Osb.) trees about 45 years old in one part of a grove at Rockledge.

Records of the occurrence of Clitocybe root rot in Lake County consist of a seedling grapefruit (*Citrus paradisi* Macf.) tree probably 45 years old and a seedling sweet orange tree in a grove at Umatilla where trees had been lost over a period of years from a root trouble attributed to foot rot, and also a seedling sweet orange tree in an abandoned grove near the south shore of Lake Apopka, west of Oakland.

The only record of Clitocybe root rot in Marion County consists of a single seedling sweet orange tree in a grove in Orange Hammock west of Altoona, although the owner previously had reported losing an occasional tree from this trouble.

In the brief opportunity to examine citrus groves in Highlands County, the writer found only a single case of Clitocybe root rot on rough lemon under Temple orange at Lake Placid. However, judging by the prevalence of oak scrub prior to clearing and the death of large numbers of horsetail beefwood (*Casuarina equisetifolia*) trees from this disease in roadside planting, it should occur frequently in the citrus groves of this section.

A systematic survey will probably show Clitocybe root rot to be of fairly widespread occurrence throughout much of the ridge section of Florida, where rough lemon stock is used generally, and probably also in sections other than those here reported. This disease was reported by the county agricultural agent to occur in groves on sweet orange stock at De Land, Valusia County, but the writer has seen no cases in the few groves he has examined.

A total of approximately 340 bearing citrus trees, nearly all on rough lemon stock, have been found attacked by Clitocybe root rot in the preliminary observations thus far made in Florida.

SYMPTOMS

The symptoms in citrus trees attacked by Clitocybe root rot vary greatly according to the progress of the disease. As a rule symptoms of decline do not develop until the disease has been working for a number of years and has killed a large proportion of the root system. A surprisingly large number of attacked trees in groves in various localities of the State have been located merely by the presence of either fresh or old, dried clusters of the fruiting bodies of *C. tabescens* at the trees bases (Fig. 1) in cases where the disease had been developing for some time but had not progressed sufficiently to cause other symptoms. Such trees usually appear as healthy and productive as adjacent nonattacked trees, though sometimes showing a general lack of vigor and a slight yellowing of the foliage. However, upon removing the soil from the root crowns and adjacent roots, a number of the lateral roots are found invaded and killed for varying distances from the root crown (Fig. 2). In most of the attacked trees examined the taproots

also were invaded and killed. The disease generally appears to start at some point on the lateral roots or adjacent to the root crown and to spread

FIG. 1. Clusters of mushrooms of *Clitocybe tabescens*. A. Old clusters at base of grapefruit tree on rough lemon stock. B. Fresh clusters at base of orange tree on rough lemon stock.

thence to other roots and the base of the tree. The distal portions of attacked roots frequently are not invaded by the fungus and remain alive

until the roots are girdled. In some cases elongated bark lesions varying
from a few inches to a foot or more in length have been found along one side
of living lateral roots. In a number of cases such lesions had become de-
limited by the formation of a callus around the periphery, in which case the
dead bark eventually disintegrated and sloughed off. In some cases a small
dead area was found at one side of the base of the trunk. Such places
frequently mark the point where a large lateral root has been killed back to
the root crown and usually are places at which one or more clusters of the
fruiting bodies of the fungus have developed.

In more advanced cases of the disease where fruiting bodies of the fungus
were not present a further symptom sometimes is a slight gumming around

FIG. 2.   Valencia orange tree on rough lemon stock attacked by Clitocybe root rot,
showing young cluster of mushrooms developing at tree base and dead bark on root crown
and roots after soil was removed.

the root crown and extending upward on the base of the trunk. The gum-
ming area of bark dies within a short time and develops small longitudinal
cracks. In still older cases the bark at the base may die on one side of the
trunk, or sometimes partly around it, and crack loose along the callus de-
veloped at the margin where the bark remained living. These basal lesions
usually extend upward from a few inches to 12 or 15 inches, and closely
resemble lesions due to foot rot. In long-standing cases of root rot the bark
of these lesions may be completely sloughed off. Not infrequently, these
basal lesions serve as points for the entrance or fruiting of secondary wood-
rotting fungi, particularly species of *Ganoderma*.

In the majority of the attacked roots of numerous citrus trees that have
been examined, the mycelium of the Clitocybe root-rot fungus was abundant,

except in those cases where it had run its course and died. The mycelium varies from thin, white to cream- or chamois-colored wefts to felty layers of extensive development between the bark and the wood and also through the inner layers of the bark. Such mycelial sheets often are characterized by a more or less radiating or fan-shaped marginal development and a distinctly perforate effect. Where the margin of the mycelium is invading living roots a slight gumming may occur in the cambial region (Fig. 3). Freshly dug roots in which the mycelium of this fungus is still active have a pronounced mushroom or fungus odor.

In citrus trees the mycelium of the Clitocybe root-rot fungus rarely extends much above the ground-line, although it usually develops upward for distances ranging from a few inches to a foot or two in other attacked trees. Mycelium of this fungus has been found occasionally, however, between the
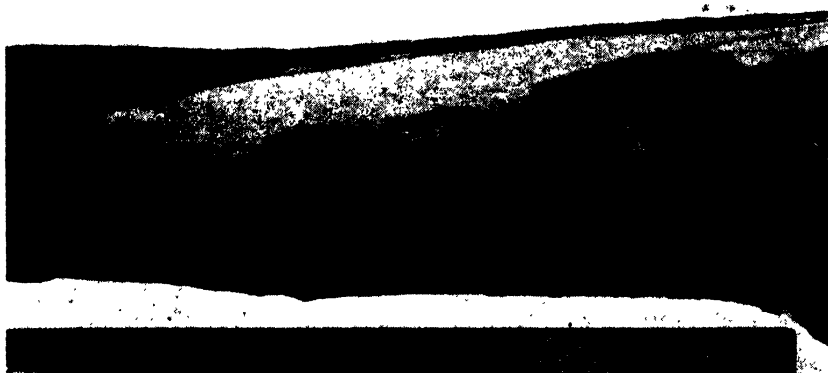
FIG. 3. Living root of old seedling sweet orange tree attacked by Clitocybe root rot, with bark cut away, showing radiate development of invading mycelial sheet and gum formation in cambial region.

bark and the wood in actively growing localized lesions at one side of attacked citrus trees. As a rule, though, it is rarely possible to diagnose this disease on citrus trees by the simple expedient of making a cutting test of the bark at the base to reveal the presence of mycelial sheets, as usually can be relied upon in other trees if they have been attacked long enough for the mycelium to extend upward between the bark and the wood above the ground-line.

Both radial and concentric cracks often appear in the wood of the larger attacked roots and become filled with whitish, felt-like layers of mycelium. In several instances even the wood in the butt of the tree has been invaded and heart rot has resulted, so that occasionally trunk divisions of large attacked trees split apart. In well-advanced cases of the disease the fungus causes a soft, whitish, delignifying type of decay of the deeper-lying roots and the decayed wood usually becomes watersoaked, soft and spongy, and often distinctly gelatinous in the late stages of decay. Longitudinal sections of such extensively decayed roots frequently show extensive pockets,

with whitish strands of mycelium in the interior, honeycombing the wood (Fig. 4).

The black, rounded or flattened, cortical and hypogeal, string-like rhizomorphs so frequently accompanying the closely related Armillaria root rot have never been found associated with root rot caused by *Clitocybe tabescens*, either in citrus or other trees. In both citrus and other trees attacked by *Clitocybe*, however, peculiar blackish, indurated xylostroma outgrowths, resembling the stromata of certain Pyrenomycetes, frequently develop through longitudinal fissures in the bark of attacked roots (Fig. 5). These extrusions do not develop in all cases but develop profusely in some.

FIG. 4. Longitudinal section through division of taproot of seedling sweet orange tree attacked by Clitocybe root rot, showing soft, spongy, delignifying type of decay and honeycombing of wood.

They may develop on both small and large roots but are much larger and more prominent on large roots, where they may be $\frac{3}{16}$ inch wide and several inches long. Such structures also occur more or less commonly in the root rot caused by *Armillaria mellea*.

In the case of a single seedling grapefruit and a few seedling sweet orange trees attacked by Clitocybe root rot, on roots with abundant xylostromata there also was noted black, bristle-like rhizomorphic structures protruding a short distance beyond the bark at right angles to the growth axis of the roots. These structures have been noted in but very few cases. Judging by their fineness, they would be very fragile when the soil becomes dry and would also be rather short-lived. The remains of these structures are apparent as black specks studding the bark between the network of

xylostroma extrusions (Fig. 5). In the case of a single old seedling grapefruit tree attacked by Clitocybe root rot at Umatilla, on a section of one root that was removed, a few scattered, flabby, black rhizomorphic structures were observed on the under side, extending downward into the soil. These had a maximum length of approximately half an inch.

By the time the Clitocybe root-rot fungus has invaded a portion of the root crown and sufficient of the roots to interfere with the maintenance of the top of the tree, the tree declines rapidly. The foliage becomes pallid and somewhat smaller than normal and the leaves become chlorotic along the midribs, as is invariably the case with acute starvation of citrus trees by any form of girdling or root-killing. The most seriously affected branches die back rapidly and the fruit produced on these devitalized branches is small and frequently drops before reaching maturity because of
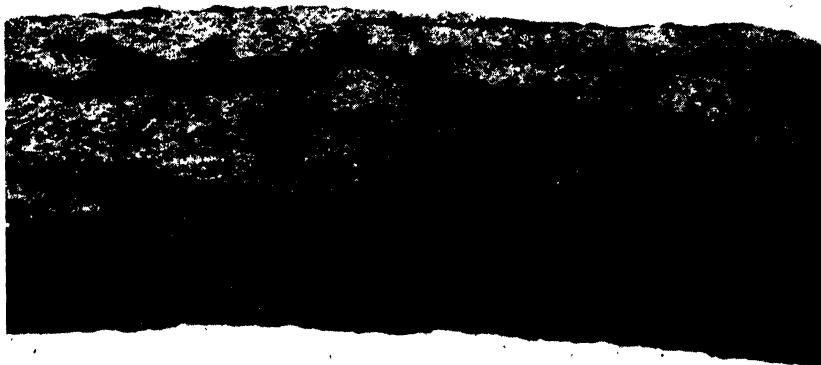
FIG. 5. Root of old seedling grapefruit tree attacked by Clitocybe root rot, showing blackish xylostroma outgrowths developed through fissures in the bark and the remnants of small, bristle-like, rhizomorphic structures protruding through the bark at right angles to it. Natural size.

the decline of the top. In the spring such declining trees develop an abnormally heavy bloom, which is also a characteristic feature of girdled or root-injured trees. In some cases where the bases of attacked trees become extensively girdled by the fungus the entire top may wilt and die rather rapidly. In some instances trees that have been greatly weakened by the rotting of a number of roots may be blown over by high winds.

<div align="center">CAUSE</div>

The root-rot disease under discussion is caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres., one of the gill fungi. The fruiting bodies of this fungus consist of a few to many individuals with the stems developing from a common base. When fully developed, the caps are convex to flattened or centrally depressed with age, whitish to light tan- or honey-colored, smooth or adorned with tufts of fibrils near the center, and from 2 to $3\frac{1}{4}$ inches in diameter, with whitish gills underneath. The united *Clitocybe*, as this

fungus commonly is called, resembles the closely related honey agaric (*Armillaria mellea*) in habit of growth, color, texture, and general appearance but is distinguished principally by the complete lack of an annulus, dissimilar spores, and in being more slender from the beginning. *Armillaria* is of cosmopolitan occurrence and causes a very similar root rot. A comparative cultural study of these two closely related root-rot fungi has been made (19). *A. mellea* occurs more or less frequently in parts of northern Florida but in other parts of this State it is largely supplanted by *C. tabescens*. The mushrooms of the latter fungus usually attain their full development within a few days from the time the young "buttons" appear and decay rapidly in warm, rainy weather. However, if dry weather follows their development the clusters may dry and turn dark brown to blackish but remain recognizable for several weeks.

The presence of either fresh or old clusters of the fruiting bodies of the fungus frequently enables one to locate attacked citrus trees long before the presence of the disease is even suspected. In groves where Clitocybe root rot is known to occur a periodic inspection for the fruiting bodies of the causal fungus often will enable the grower to locate attacked trees and treat them before the disease kills the roots to such an extent that it becomes difficult or impossible to save the trees.

The production of fruiting bodies of the fungus depends largely upon the progress of the disease and the seasonal conditions. They develop with greatest frequency during the fall and early winter, from about mid-September to mid-December, but may develop in other months as well if soil moisture is favorable. As a rule only one or two clusters develop during the year and frequently none develop.

Origin and Anatomy of Xylostroma Outgrowths in Bark of Roots

The structure of the xylostromata that frequently develop as hard, blackish, stromalike structures extruded through more or less longitudinal cracks in the bark of roots of woody plants attacked by *Clitocybe tabescens* is essentially identical with that of the outgrowths of *Armillaria mellea* described and illustrated by various writers. The structure of the mycelial sheets of *Armillaria* as they occur under the bark was described by Hartig (5) as similar to that of the rhizomorphs except for modifications due to their different form and environment. Their occurrence in connection with the "collar crack" disease of cacao (*Theobroma cacao*) caused by *Armillaria* was described and illustrated by Dade (2). In the Gold Coast form of this disease on cacao in Africa, however, he found mostly a meager development of xylostromata between the bark and the wood but a strong tendency for development of xylostromata in the prominent medullary rays of the wood, which resulted in radial splitting of the stems. He designated the more or less frilly outgrowths of these mycelial sheets through longitudinal fissures in the bark as xylostroma extrusions.

The writer has studied the development of these xylostroma outgrowths

in cultures of both these root-rot fungi in large test tubes and jars containing lengths of woody stems or roots with the ends in agar in the bottom of the tube or jar. When the growth of the mycelial sheets between the wood and the bark becomes sufficient to cause the bark to rupture longitudinally they grow out, sometimes in frilly fashion, through the fissures thus formed. Distention of the bark by continued growth and thickening of the xylostromata results in further widening of these fissures. When freshly extruded, these xylostroma outgrowths are soft and whitish, but following exposure to air and desiccation, as occurs in nature, they soon develop a brittle, horny, dark-brown to blackish crust, like old rhizomorphs, in which grains of sand are firmly imbedded (Fig. 5). That these structures are merely
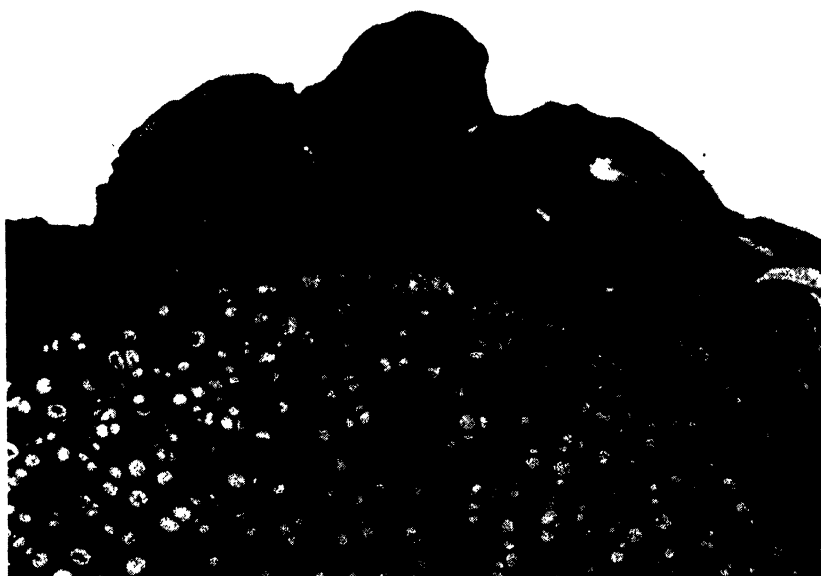
FIG. 6. Transverse section of small root of rough lemon under Marsh grapefruit attacked by Clitocybe root rot, showing xylostroma formation extruded through longitudinal fissure in bark. × 30.

outgrowths of the mycelial sheets developed between the bark and the wood, extruded through longitudinal fissures in the bark, is clearly shown by a study of transverse sections of the invaded roots (Fig. 6). These xylostroma outgrowths have been regarded as rhizomorphs by Darnell-Smith and Mackinnon(3, Fig. 2), Birmingham (1, Fig. 2) and Fawcett (4, Fig. 5, B) in their discussions of Armillaria root rot of citrus, each using the same illustrations. Although these xylostromata are comparable structurally with rhizomorphs, they are not to be confused with the usual shoestring-like rhizomorphs.

ISOLATIONS AND PATHOGENICITY OF THE CAUSAL FUNGUS

In view of the fact that *Clitocybe tabescens* has been isolated consistently

from the roots of a diverse array of plants attacked by root rot in various parts of Florida over a period of several years, only sufficient isolations were made from citrus trees to demonstrate that this fungus was consistently associated with trees attacked by root rot. From 1929 to 1935 isolations were made from the roots of 10 different living citrus trees attacked by root rot at various localities in Polk and Brevard Counties, and in each case pure cultures of *C. tabescens* were obtained. Two of these isolations were from the roots of rough lemon under grapefruit, respectively at Lake Alfred and Highland City, Polk County. Of the others, which were from Brevard County, three were from roots of rough lemon under grapefruit at Georgiana, Merritt Island, one from roots of rough lemon under cala-mondin at Melbourne, and three from roots of old, seedling sweet orange trees at Rockledge. All but two of these isolates developed fruiting bodies in test tube or flask cultures.

From 1924 to 1946 Clitocybe root rot has been recorded in Florida as attacking 210 different species of plants belonging to 137 genera and 59 families. The writer has isolated the causal fungus from the roots of 156 plants comprising 90 different species. These comprised native forest trees, tungoil trees, ornamental trees, shrubs and vines, and fruit trees, including banana, citrus, guava, and various other subtropical ones. Although no op-portunity has been afforded to inoculate bearing citrus trees with *C. tabes-cens,* the repeated finding of this fungus attacking the root systems of living trees in various groves and its frequent and widespread occurrence and destructive effect on a diverse array of other trees, shrubs, and vines leaves no doubt that it is a virulent parasite capable of attacking uninjured roots of vigorously growing plants and spreading rapidly to adjacent ones, especially when closely planted. The writer (20) has shown it to be particularly destructive to plantings of beefwood (*Casuarina spp.*) trees, or so-called Australian pines, in Florida and has reported (15, 16, 17) successful in-oculation experiments with it on both the horsetail beefwood (*C. equiseti-folia*) and the scalybark beefwood (*C. lepidophloia*). Plakidas (6) also reported the same with young Pineapple pear trees.

RELATION OF INCIDENCE OF THE DISEASE TO SOIL CONDITIONS AND TIMBERED LANDS

Clitocybe root rot of woody plants in Florida occurs on a variety of soil types, being rather frequent on the lighter, well-drained sandy soils, which are dominantly acid in reaction and inclined to be more or less droughty. However, it also occurs on soils with an alkline reaction. In citrus trees the disease thus far has been found on but a few soil types. In the central part of the State it has been found almost exclusively on Norfolk sand, while in the few groves in Brevard County on the East Coast it occurred largely on Norfolk fine sand, though on Gainesville sand (coquina phase) in the grove at Rockledge. However, the soil type in general appears to have less influence on the incidence of the disease than has the prevalence of oak and other hardwood trees on the land prior to clearing.

The incidence of Clitocybe root rot of citrus trees in a number of groves in various parts of Polk County has been found correlated with the use of timbered land for planting. The tree growth prior to clearing was the characteristic pine-oak association, with a hammock type of growth in some cases. In practically all the 77 trees dug around for surgical treatment late in 1930 and early in 1931 in 6 groves at Lake Alfred, oak and occasionally other hardwood roots infected by *Clitocybe* were found. These citrus groves were from 9 to 19 years old and the roots that had been left in the land when it was cleared, though certainly by no means all infected at the time, enabled the fungus to spead and served to transmit it to the roots of the citrus trees with which they came into contact. It also was observed in several instances that where an infected citrus root crossed or came into contact with another citrus root that the root-rot fungus was transmitted to this root.

In the grove at Kathleen, where Clitocybe root rot was reported occurring first about 1915, it was related by the former owner that when the land was cleared in 1910 it contained numerous bluejack and turkey oaks (*Quercus cinerea* and *Q. laevis*) and that a good many stumps were left in the ground for three or more years after the trees were planted. Oak roots infected by the root-rot fungus also were found under a number of citrus trees attacked by Clitocybe root rot in one grove at Highland City. It therefore appears that oak and other hardwood roots left in the land at the time of clearing can constitute sources of infection in planted citrus trees for a greater number of years than is generally believed.

However, not all cases of Clitocybe root rot in citrus groves have been correlated with the spread of the fungus from infected roots in the land. In two groves at Georgiana on Merritt Island, where a considerable number of attacked trees were dug around for surgical treatment, oak roots were almost completely lacking, only a single small infected piece being found. However, uncleared land with oak trees adjoined the attacked trees in one grove. Moreover, in the grove at Rockledge no evidence of foreign roots was found under 4 seedling sweet orange trees, probably close to 45 years old, that were given surgical treatment. It therefore appears that infection by this disease must at times occur in some way other than by contact with infected roots, probably resulting from infection by the spores of the fungus.

COMPARATIVE SUSCEPTIBILITY AND RESISTANCE OF DIFFERENT ROOTSTOCKS

With the exception of a few old seedling sweet orange trees, an old seedling grapefruit tree, and those trees on everbearing lemon stock at Kathleen, all the citrus trees found attacked by Clitocybe root rot have been on rough lemon stock. A total of approximately 325 trees on this stock have been found attacked. Approximately two-thirds of these were budded to grapefruit, nearly one-third to orange, 9 to tangerine, 1 to calamondin, and 1 young tree to Tahiti lime.

Only in the 20-acre grove at Kathleen, which is unusual in that a number of rootstocks were used, has an opportunity been afforded to make a comparison of the incidence of Clitocybe root rot on different rootstocks in a property where this disease is known to have occurred for several years. A detailed inspection was made of this grove in June, 1935, following the receipt of the history and a diagram of the varieties and rootstocks used in the different parts, which were kindly furnished by the former owner, Mr. W. E. Tucker. Clitocybe root rot was reported to have developed about 1915 but, aside from half a dozen declining trees, the grove was said to have been in fine condition when sold in 1918. However, Mr. Tucker, who visited his home adjacent to the grove at intervals, reported that since then there had been considerable loss of trees from both root rot and the chronic wilt and decline of citrus trees known as "blight."

The trees purchased for the west three-fourths of this grove were supposed to have been budded on rough lemon stock but a considerable number actually were on everbearing lemon, as evidenced by the fruit borne on root sprouts that developed. It is well known that seed of this variety of lemon was used formerly by some nurserymen and that trees on this stock have proved short-lived and subject to "blight." Tucker stated that his trees on this stock proved unusually susceptible to both Clitocybe root rot and "blight."

In the 10-acre block of orange and grapefruit trees on the west half of this grove in which lemon (rough and everbearing) was used as the stock a large number of trees had been removed, while others had been braced with guy wires following extensive surgical treatment. As many as 17 of the original trees in one row had been either treated or removed. Immediately east of this 10-acre block were two adjoining small blocks of grapefruit trees, one being on mixed lemon stock and the other on grapefruit stock. In the former a heavy loss of trees was apparent, whereas no evidence of any tree loss occurred in the latter. This striking difference indicates a marked resistance of the grapefruit stock under conditions where the rough and everbearing lemon stocks proved highly susceptible. On the east side of this grove 4 rows of Valencia oranges on grapefruit stock were free from tree losses. In the balance of the east side of this grove, which was planted to Ruby orange on a mixture of rough lemon and sour orange stock, some losses occurred in trees on the former stock but none was apparent in those on sour orange. The complete freedom from root rot of the trees on sour orange stock in this block indicates a high degree of resistance, a characteristic that has been verified for this stock by general observations throughout a large part of the citrus region of Florida.

Although numerous groves have been examined in various sections of the State, not a single case of Clitocybe root rot has been observed on trees on sour orange stock, including the bittersweet which is botanically the same species and often used indiscriminately in mixture. No instance has been observed of citrus trees on sour orange stock being attacked by root rot,

even in groves on Merritt Island, where the disease often causes a heavy mortality of beefwood (*Casuarina spp.*) trees, or so-called Australian pines, in windbreaks planted closely around and through the groves and occasionally interplanted between the citrus trees in the same rows. Judging from these observations, trees on sour orange stock possess a high degree of resistance to Clitocybe root rot, as they do to a number of other diseases. Citrus trees, in general, appear to be much more resistant to this disease than many other woody plants.

TREATMENT OF ATTACKED TREES

It has been not only possible but quite practicable to save, by careful surgical treatment, bearing trees that are attacked by Clitocybe root rot, provided the disease be located and treatment administered early. The extent to which the disease has attacked the root system can be determined only by removing sufficient soil from around and under the root crown to permit a thorough examination. This requires removing the soil in a basin with a radius of at least from 2 to 3 feet from the trunk, working carefully to avoid injuring living roots. Unless facilities are available for washing away the soil hydraulically it should first be removed from around the base of the tree with a trowel until the location of the lateral roots can be determined. One half of a post-hole digger makes an excellent implement to remove the soil from around the root crown under the lateral roots when they are too close together to use a shovel.

If it is found practicable to treat the tree, all dead roots should be cut off flush with the root crown or living roots from which they branch, and removed in their entirety. Special effort should be made to remove any infected oak or other foreign roots that may be found under the trees being treated. All bark lesions that may occur at the base of the trunk or on the root crown, lateral roots, or taproot, in case the latter is not dead, should have the dead or infected bark cut out carefully back to healthy bark and down to sound wood, using a chisel and gouge with a mallet. The lateral roots should be inspected carefully for local lesions, particularly at their juncture with the root crown. All roots and chips of wood and bark excised should be removed and burned, because allowing such infected material to remain in the soil may lead to the redevelopment of the disease. A heavy paper placed under the exposed roots greatly facilitates the collection and removal of such material. All trees not worth treating should be dug out, and pains taken to remove and burn all roots.

After the surgical treatment, the treated areas should be brushed clean of soil and the roots inspected for any lesions that may have been overlooked. All exposed wood surfaces should then be painted with a good, durable pruning-wound compound. After this has dried, the excavation may be filled partially or completely. If the tree is not banked to stimulate the development of new roots, it is a good idea to leave the root crown ex-

posed indefinitely for aeration and drying. The soil may be disinfected with 2 to 3 gallons of a strong Bordeaux or caustic soda-Bordeaux mixture (5-5-50) poured into the soil basin. If the tops of the treated trees have died back they should be dehorned proportionately. The location of the treated trees should be mapped and they should be inspected periodically.

Frequently, the mere removal of the soil to expose the root crown and adjacent lateral roots to aeration and drying will prove very effective in checking the further development and spread of the disease on the attacked tree because the growth of the fungus is arrested by desiccation. Extensive surgical treatment of trees sometimes leads to decay of the exposed wood at the base of the trunk despite the use of a pruning-wound compound. The recovery of trees that have lost a considerable proportion of their root systems can be expedited greatly by banking or mounding the soil around the bases up to a height of several inches above the upper limit of the partial girdle to stimulate the development of new roots from the callus formation formed at the margin of the living bark. This little-known natural method of providing partially girdled trees or those deprived of many of their roots with new root systems (18) has proved of considerable value in citrus and other trees and shrubs that develop adventitious roots readily. Under favorable soil moisture conditions new roots start developing in a few months and within a year or two should attain sufficient growth to contribute materially to the support of the trees and assist in their rejuvenation.

The writer has secured very promising results from the surgical treatment of 123 citrus trees attacked by Clitocybe root rot. Success is contingent upon careful treatment and aeration of the root crown. Treatment, of course, must be administered before the disease has progressed to such an extent that the trees begin to decline. A total of 77 trees in a number of groves at Lake Alfred were treated late in 1929 and early in 1930. With the exception of one that blew over shortly afterward, all have continued to flourish and bear good crops of fruit. Thirty-six trees, including a considerable number that had begun to decline, were treated in a grove at Georgiana, Merritt Island. Of these, 20 were still living and in good condition in September, 1941; the others died and were removed at various times. Four old, seedling sweet orange trees, three of which were treated in 1931 and one in 1933 at Rockledge, and six grapefruit trees treated in 1933 at Georgiana, have all continued to thrive and were bearing good crops in September, 1941. These results show that a single surgical treatment, without soil banking in conjunction, has proved extremely effective in controlling Clitocybe root rot and in adding a considerable number of years to the life of bearing trees attacked by it in practically all cases where the trees were not too far gone when treated. Similar beneficial results have been observed in a number of trees treated surgically according to the writer's recommendations by one grower at Highland City and another at Winter Haven.

DISCUSSION

As was pointed out previously (11, 12), it has long been the custom in Florida to diagnose all decline and death of citrus trees in which the trouble was characterized by a basal girdling and death of the roots and sometimes accompanied by an exudation of gum from the bark, as foot rot (*Phytophthora parasitica*). This appears to have resulted largely from the great publicity formerly given this disease and its prevalence on seedling sweet orange trees and trees budded on this stock, which are highly susceptible to *Phytophthora*. Contrary to popular opinion, rough lemon stock has never proved particularly susceptible to foot rot. Much of the trouble that formerly was assumed to be foot rot has been shown by these investigations to be due to Clitocybe root rot instead. This disease is especially prevalent on trees on rough lemon stock and it also occurs on seedling sweet orange trees. Thus, the recognition of Clitocybe root rot and its evaluation as an important cause of tree loss in citrus groves in various parts of Florida enables us to properly diagnose this disease rather than to continue to arbitrarily assign to foot rot all diseases that girdle the trees.

SUMMARY

Mushroom root rot, caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres., is discussed and evaluated as a factor of tree loss in Florida citrus groves. Although not identified as occurring on citrus trees until 1929, it is evident that this disease was active in groves several years before but that it previously was confused with foot rot, the secondary symptoms of which have a similar appearance.

A record, based on a preliminary survey, is presented of the known distribution of Clitocybe root rot on citrus trees in various counties of the State, and of isolations made of the causal fungus.

The symptoms of the disease are described in detail. Its attack usually is concentrated chiefly on the root crown and adjacent portions of the root system. It frequently results in the death of the taproot and a large proportion of the lateral roots, and sometimes causes a butt rot of the trunk before the top shows any appreciable evidence of decline. Many trees not even suspected of being attacked, but subsequently found to have their root systems invaded extensively, have been located solely by finding either fresh or dried clusters of the causal fungus fruiting at the bases. The xylostroma outgrowths frequently developing on attacked roots are described and their origin and anatomy discussed.

Clitocybe root rot occurs with greatest frequency on well-drained, and often droughty, sandy land, especially in sections where oak and other hardwood trees occurred prior to clearing. Roots of these trees attacked by the Clitocybe root-rot fungus have been found repeatedly under attacked citrus trees in groves ranging in age from 9 to 19 years. Infection in many cases results from the roots coming into contact with infected roots but the failure

to find foreign roots under trees in many cases suggests that infection may be initiated by some other means, presumably from the spores of the fungus.

Most of the citrus trees found attacked by Clitocybe root rot have been on rough lemon, with some on everbearing lemon, stock. Both are very susceptible. Seedling sweet orange trees are also susceptible, while the little used grapefruit stock appears fairly resistant and the sour orange stock very resistant. Citrus trees in general appear to be much more resistant to this disease than many other woody plants.

The treatment of attacked trees is discussed and marked success reported with surgical treatment, either alone or in conjunction with exposure of the root crowns to aeration and drying. The value of subsequently mounding the soil around the bases of treated trees to stimulate the development of new root systems is pointed out.

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THE IDENTIFICATION AND CHARACTERIZATION OF A VIRUS CAUSING MOSAIC IN MERTENSIA VIRGINICA

INEZ NIENOW [1]

Johnson and Valleau in 1935 (10) reported a virus from *Mertensia virginica* (L.) DC. which could be transferred to Turkish tobacco producing chlorotic and necrotic rings on old inoculated leaves and a mottle similar to cucumber mosaic on new leaves. In 1946 *M. virginica* plants with mosaic symptoms were found at Urbana, Illinois. *M. virginica,* the common bluebell, is a perennial and widely distributed. This host, therefore, could serve as an important source of infection for susceptible plants. Since the cause of the disease appeared to be cucumber mosaic virus which infects several economic crops, the following group of studies was undertaken to identify and characterize the virus causing the Mertensia mosaic.

SAP INOCULATION

Inoculations were carried out with crude infectious juice from *Nicotiana glutinosa* L. and *N. tabacum* L. (Turkish). Two to four young leaves of the plant were dusted with carborundum of 600 mesh prior to being rubbed once with gauze pads saturated with infectious juice. Inoculated leaves were washed immediately to remove any toxic effects of the juice. For the quantitative studies on the effect of heat, pH, and phosphate buffer on the virus, primary leaves of blackeye cowpea which gives local necrotic lesions were used. The right half was inoculated with the variant and the left half with the control. Ten half-leaves were used for each sample. For tests on aging of the virus, whole cowpea leaves were inoculated. Local lesions were counted four days after inoculation.

INSECT INOCULATION

Aphids[2] were allowed to feed on caged *Nicotiana glutinosa* plants that were infected with Mertensia-mosaic virus. After seven days of feeding, the aphids were transferred to two healthy *N. glutinosa* plants and one *N. repanda* and allowed to feed for seven more days. These plants were screened from other insects by means of a lamp chimney covered with cheesecloth. *Myzus persicae,* after feeding on infected *Nicotiana glutinosa* plants, transmitted the virus to healthy *N. glutinosa* and *N. repanda* plants. These

developed symptoms of necrosis, chlorosis, and mottling typical for the virus on these plants.

HEAT INACTIVATION

One ml. of infectious crude juice from petunia plants was placed into 3-inch serological tubes and stoppered with cotton. A control tube of 1 ml. of unheated infectious juice was prepared for each heated tube of juice. The tubes were placed in a steam-controlled agitated water bath and heated to the various desired temperatures. After a 10-minute period the tubes were quickly cooled by placing them in cold running water. All inoculating onto cowpea leaves was done at one time and within two hours after the juice was heated. The results from duplicated experiments (Table 1) indicate that the virus is inactivated somewhere between 65° C. and 70° C. for ten minutes, but that temperatures as low as 50° C. reduce infectivity.

TABLE 1.—*Effect of heat on infectivity of Mertensia-mosaic virus in crude juice*

Temperature	Mean number of local lesions on a half-leaf of cowpea	Standard deviation
50° C.	1.90	2.308
Control	7.40	4.710
55° C.	0.45	0.203
Control	8.80	6.982
60° C.	0.55	0.780
Control	8.10	5.996
65° C.	0.05	0.020
Control	10.70	7.736
70° C.	0.00	....
Control	9.40 .	8.192
75° C.	0.00	.. .
Control	6.70	3.471

pH AND PHOSPHATE BUFFER

Infected petunia plants were ground and the juice squeezed through four thicknesses of cheesecloth. This juice was adjusted to pH 5.5, 6.0, 7.5, 8.0, 8.5, 9.0 using KH_2PO_4 or K_2HPO_4 and 0.1 N NaOH so that the final salt concentrations were 0.1 M. phosphate. One ml. of juice was added to 9 ml. of buffer so that all data represent a 1–10 dilution of the infectious juice. Inoculations were completed within 1 hour following pH adjustments at room temperature. Concentrations of H-ions and of phosphate buffer salts influence the infectivity of tobacco-mosaic virus (16). Since 0.1 M. salt concentration was optimal (16), only this concentration of the buffers was used. From the results in table 2, it is apparent that 0.1 M. phosphate buffers increase the infectivity of the virus and that pH 6.5 is the most desirable pH for inoculation of the virus onto cowpea leaves.

AGING

Two ml. of infectious juice were placed into 3-inch serological tubes and kept at room temperature of 25°–28° C. for 1, 2, 4, 8, 12, or 48 hours prior

TABLE 2.—*Effect of pH and 0.1 M. phosphate buffers on production of local lesions on cowpea leaves by Mertensia-mosaic virus*

pH	Mean number of local lesions on a half-leaf of cowpea	Standard deviation
5.5	5.3	3.234
Control (6.7)	2.3	1.421
6.0	6.5	5.788
Control	1.7	1.519
6.5	18.2	12.569
Control	2.0	2.024
7.0	7.9	8.026
Control	2.1	2.121
7.5	2.2	1.772
Control	1.2	0.242
8.0	3.9	6.074
Control	1.6	2.061
8.5	1.3	2.439
Control	1.9	2.737
9.0	3.4	2.505
Control	2.7	3.142

to being inoculated onto the cowpea leaves. In order to evaluate the time in which it was possible to maintain optimal infectivity, the effect of aging of the virus in expressed juice was determined. The results in table 3 indicate that the host plant may influence the length of time infectious juice retains its infectivity; they also show the need for working with the virus as quickly as possible to avoid infectivity changes due to aging.

SEROLOGICAL AND CROSS PROTECTION STUDIES

In this study crude infectious juice of the petunia plant which had a consistently high concentration of virus was used as a source of antigen for eight intraperitoneal inoculations of 5 ml. each into the rabbit. Antiserum was obtained by the usual procedures. In setting up titers for flocculation tests, the antigen rather than the antiserum was diluted. Tubes were incubated for two hours at 37° C. in a water bath, and then kept overnight in the refrigerator. Initial readings were taken after incubation and final readings after refrigeration. The possibilities of serological studies as a means for plant virus identification has been demonstrated by Beale (2), Birkeland (3), Chester (4, 5, 6, 7), Gratia (8, 9), and Purdy (14, 15). Titers were

TABLE 3.—*Effect of aging at 25° C. upon the infectivity of Mertensia-mosaic virus in crude juice*

Source of virus	Total number of local lesions on four cowpea leaves, time in hours							
	0	1	2	4	8	12	24	48
Leaves, stems of petunia	17	35	31	14	7	6	0	0
Leaves of *Nicotiana sylvestris*	56	32	4	7	0	0	0	0
Leaves of *N. tabacum* (Turkish)	63	6	5	1	0	0	0	0
Leaves of *N. tabacum* (Burley)	17	52	19	23	7	......	7	0

demonstrated against the Mertensia virus in tobacco, petunia, and cucumber as well as against *Cucumis virus*[3] in tobacco. In this study titers were obtained with absorbed antiserum of 1–128 with *Cucumis virus* in *Nicotiana glutinosa*, 1–64 with Mertensia mosaic virus in petunia, 1–32 with Mertensia-mosaic virus in cucumber. The higher titers with the *Cucumis virus* in tobacco were probably caused by a higher concentration of virus in the plant. That the virus occurring on *M. virginica* was cucumber mosaic virus was further substantiated by cross protection tests. Price (12, 13) has shown that zinnia leaves infected with a strain of cucumber-mosaic virus will not thereafter develop the necrotic lesions caused by strain 6 of cucumber-mosaic
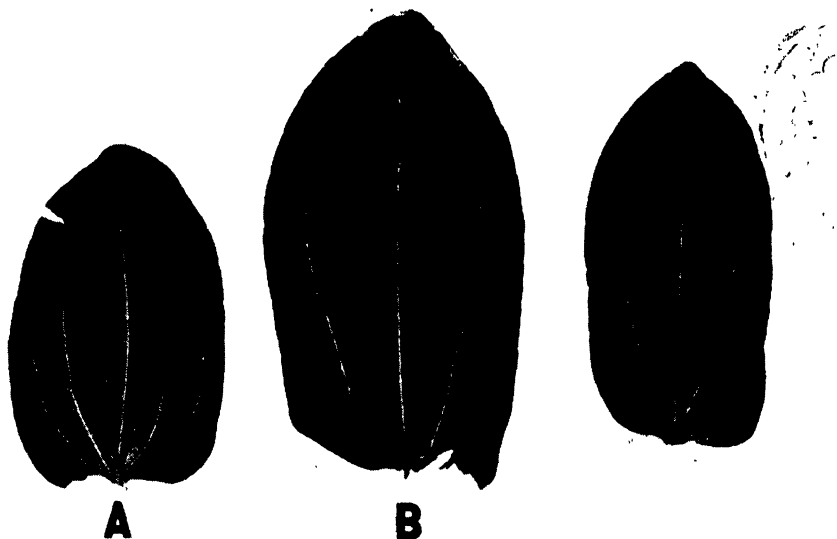
FIG. 1. Cross protection test with related strain of cucumber mosaic virus, using strain 6 as the indicator strain which gives local necrotic lesions on zinnia leaves when the leaves have not been previously infected with a cucumber-mosaic virus: A. Infected with Mertensia-mosaic virus followed by inoculation with strain 6 cucumber-mosaic virus. B. Infected only with strain 6 cucumber-mosaic virus. C. Infected with tobacco-mosaic virus followed by inoculation with strain 6 cucumber-mosaic virus.

virus. Necrotic lesions will develop on non-infected leaves or on leaves infected with viruses other than cucumber mosaic.

When non-infected zinnia plants or zinnia plants infected with tobacco-mosaic virus were inoculated with strain 6 (indicator strain) of cucumber-mosaic virus,[4] both developed local necrotic lesions. Zinnia plants inoculated with Mertensia-mosaic virus and later with cucumber-mosaic virus strain 6 did not develop any necrotic lesions, but only showed the mosaic

[3] Obtained from Dr. S. P. Doolittle, U.S.D.A., Bureau of Plant Industry, Beltsville, Maryland.
[4] Obtained from Dr. W. C. Price, University of Pittsburgh, Pittsburgh, Pa.

from the first inoculation of Mertensia-mosaic virus (Fig. 1, A), indicating this virus is a cucumber-mosaic virus.

OVERWINTERING OF VIRUS

Diseased Mertensia plants growing in the field showed mosaic symptoms

TABLE 4.—*Summary of symptoms observed on various plants inoculated with Mertensia-mosaic virus and the results of inoculating cowpea with the juice from the respective plant species and varieties*

Species and variety of plant inoculated	Symptoms observed	Production of local lesions on cowpeas
Antirrhinum majus L.		
Golden Queen	Distortion, stunting, early flowers	+
Beta vulgaris L.		
Asgrow Wonder	Questionable	−
Early Wonder	Do	−
B. vulgaris var. *cicla* Moq.		
Spinach leaved	Systemic mottling, necrosis	+
Brassica oleracea var. *caulorapa* DC.		
Early white Vienna	No symptoms	−
B. rapa L.		
Early Purple Globe	No symptoms	−
Calendula officinalis L.		
Masterpiece	Systemic mottling	+
Cucumis sativus L.		
Cubit	Systemic mottling	+
Straight Eight	Do	+
Datura stramonium L.	Chlorotic spots on inoculated leaves, systemic mottling later	−
Delphinium ajacis L.		
Base Branching	Stunting, fern leaves, deformed flowers	+
Lactuca sativa L.		
Black Seeded Simpson	No symptoms	.....
Lycopersicon esculentum Mill.		
Prichard	Fern leaves, mottling	+
Stokesdale	Do	+
Mertensia virginica DC.	Systemic mottling	+
Nicotiana glutinosa L.	Necrosis, stunting, systemic mottling	+
N. repanda Willd.	Do	+
N. sylvestris Speg. and Comes.	Do	+
N. tabacum L.		
Burley, Ky.–16	Do	+
Burley, White	Do	+
Crittenden	Do	+
Turkish	Do	+
Petunia hybrida		
Dwarf Hybrid	Systemic mottling	+
Phaseolus vulgaris L.		
Burpee's Stringless Green Pod	No symptoms	.....
Early Golden Cluster	Do	. .
Red Kidney	Do	.. .
Tendergreen	Do	.. .
Rumex occidentalis S. Wats.	Do	−
Spinacia oleracea L.		
Bloomsdale Savoy	Necrosis, stunting	+
Tagetes patula L. (Hybrid)		
French Marigold	Necrosis of stem and petiole	+
Vigna sinensis Endl..		
California Blackeye	Primary necrotic lesions	+
Blackeye	Do	+
Zinnia elegans Jacq.	Mottling, distortion	+

the following spring. This indicated that the virus persisted in the perennial, tissues of the plant during the winter months and appeared, subsequently, in the above ground tissues where the virus became subject to spread by feeding insects to additional plants.

SYMPTOMS PRODUCED BY MERTENSIA MOSAIC VIRUS

As one means of identification, the virus was inoculated into a wide range of plants in order to compare the symptoms produced with symptoms of other viruses in these hosts. The results are summarized in table 4.

INCLUSION BODIES

Epidermis from infected leaves, stems, and petioles was stripped from infected *Nicotiana tabacum* (Turkish) and *N. glutinosa* and stained for plant virus inclusion bodies according to the methods used by McWhorter (11) with modifications suggested by him.[5] No inclusion bodies were found, which suggested that the virus is one that does not produce inclusion bodies. According to Bawden (1), cucumber viruses 1, 3, 4, and the viruses of potato leaf roll, potato paracrinkle, tomato spotted wilt, and tomato big bud do not produce intra-cellular inclusions.

SEED TRANSMISSION

Seeds obtained from infected *Nicotiana glutinosa, N. repanda,* and *N. tabacum* (Turkish) were sown and the seedlings examined for evidence whether the virus was seed transmitted. Among approximately 100–150 seedlings of each of the three kinds of tobacco, no diseased plants developed.

DISCUSSION

In the early studies, during September through December, 1946, it was difficult to transmit Mertensia-mosaic virus to plants of any variety or species because the temperature of the greenhouse fluctuated from 60° to 75° F. When the plants were moved to a greenhouse in which the temperature was maintained at 70°–74° F., symptoms of infection readily appeared on a wide range of hosts. It was noted that the virus affected the flowers of all plants which became infected: the flower buds failed to mature, fell off, or produced flowers that were small and distorted. The concentration of virus in plants, tested by the number of local lesions produced on primary leaves of the cowpea, varied considerably, *e.g.,* it was low in snapdragon and calendula, but high in petunia and some varieties of tobacco. Young plants are preferable for such studies since old plants often showed marked effects of disease yet virus concentration was not proportionately high.

Before conducting any quantitative studies, infectious plant juice was tested for concentration by inoculation onto cowpea leaves in order to give some assurance of sufficient local lesions to make such studies valid. It was apparent from the results that the Mertensia-mosaic virus rapidly lost its

[5] Personal communication dated February 14, 1947.

infectivity upon aging and that the ability to withstand aging varied with the host plant, the age of the plant, and possibly other undetermined factors.   The Mertensia-mosaic virus was rather sensitive to pH changes, and best infections were obtained between pH 6.5 and 7.0.   This pH was about that of the petunia plant but whether this was a factor in the production of high concentration of virus in this plant is conjecture.

Phosphate buffers at 0.1 M. concentration increased the number of local lesions produced.   The phosphate buffer may have released more virus from particulate material of the crude juice, or dispersed virus aggregates by increasing the solubility, or have affected the cells of the plant tissue in such a manner that infection was increased.

Since the Mertensia-mosaic virus was readily adsorbed, amenable to aging, and destroyed by freezing, it was not practical to filter or freeze the antigen before injection.   Crude, freshly extracted plant material injected immediately gave the best results.   It is advisable to determine relative amounts of virus in antigenic material that is to be used for injection into animals, since this gives assurance of an antiserum of high titer.

SUMMARY

1. The virus causing mosaic in *Mertensia virginica* (L.)DC. was identified by serological tests, cross protection tests, host range, and insect transmission as *Cucumis virus* I Smith.
2. The virus was transmissible to a large number of plants and produced a variety of symptoms dependent on the host plant.
3. The virus was inactivated between 65° and 70° C. for ten minutes.
4. Infectivity of the virus was increased by 0.1 M. phosphate buffer.
5. The most desirable H-ion concentration for inoculation on cowpea leaves was pH 6.5.
6. It was expedient to work with the virus within a two-hour period to eliminate the decrease in infectivity due to aging.
7. The virus was not transmitted by seeds of infected *Nicotiana repanda, N. glutinosa* L., *N. tabacum* L. (Turkish).
8. The virus was transmitted by *Myzus persicae* Sulzer.
9. *Mertensia virginica* (L.)DC. is a new host for *Cucumis virus* I and an important virus reservoir since it is a perennial and the virus persists in the host.

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PRODUCTION OF ANTIBIOTIC SUBSTANCES BY FUSARIA[1, 2]

DIEGO A. TEXERA[3]

HISTORICAL AND INTRODUCTORY WORK

The observation that molds of the genus *Fusarium* are able to excrete into artificial substrates metabolic products toxic to other microorganisms dates back to 1924 when Boyle and Pratt (4, 14) performed their classical studies on staling of *Fusarium* cultures.

In the field of plant pathology the antagonistic properties of fusaria have been investigated from two main angles: studies on toxic excretory substances capable of producing wilts in plants (6, 8, 13, 16, 24) and observations about their antifungal power (15, 23, 26). None of these studies was concerned with the isolation of true antibiotics of possible use in chemotherapy.

In 1937 Ashley, Raistrick, and their coworkers (3) isolated two quinone pigments, rubrofusarin and aurofusarin, and one non-pigmented substance, culmorin, from pure cultures of *Fusarium culmorum*. As far as we know, these substances have never been studied from the standpoint of antibiotics.

Still another pigment isolated from a species of *Fusarium* is javanicin, recently announced by Arnstein, *et al.* (1, 2), from *F. javanicum*. Unlike rubrofusarin and the others, the antibiotic properties of this substance have been thoroughly investigated.

Aside from this work, there have been in the past few years occasional reports concerning the ability of fusaria to inhibit the growth of other microorganisms, particularly bacteria (5, 7, 20, 25), but most of these observations have consisted of brief references incidental to extensive surveys in which the antibacterial effect of hundreds of fungal cultures was examined.

The present study is concerned with the isolation of two antibiotic substances from *Fusarium hyperoxysporum* Woll., their mode of formation, and their properties.

COMPARISON OF SAPROPHYTIC AND PLANT PATHOGENIC FUSARIA[4]
FOR BACTERIOSTATIC ACTIVITY

The introductory phase of the investigation consisted in the isolation of eighteen *Fusarium* strains from soil and composts and the examination of their bacteriostatic activity in different liquid culture media after preliminary screening by means of the cross-streak test (19). The artificial substrates compared were:

1. Glucose-peptone.
2. Glucose-asparagine (Krainsky's).
3. Glucose-nitrate (Czapek-Dox).
4. Glucose-nitrate with 0.5 per cent corn steep liquor.
5. Sucrose-nitrate (brown sugar medium).

The culture media were dispensed in 50-ml. volumes in Erlenmeyer flasks of 250-ml. capacity, neutralized, and sterilized at 10 lb. steam pressure during one-half hour. Inoculation was made with one-ml. portions of spore suspensions prepared from one-week-old slants of the strains reared in glucose-peptone agar. Incubation followed at 28° C. for two weeks and the

TABLE 1.—*Antibiotic activity of saprophytic and plant pathogenic strains of Fusarium grown in glucose-nitrate medium*

| Strain | Dilution units per 1.0 ml. at 14 days of incubation | | | | | | | |
| | Metabolic solution[a] | | | | Mycelium[b] | | | |
	E. coli	*S. aureus*	*B. subtilis*	*B. mycoides*	*E. coli*	*S. aureus*	*B. subtilis*	*B. mycoides*
Fusarium sp. 21	< 5	< 5	90	90	< 30	400	1,000	2,000
Fusarium sp. 25	< 5	< 5	80	150	< 30	125	450	450
Fusarium sp. 26	< 5	< 5	50	150	< 30	125	1,250	1,250
Fusarium sp. 30	< 5	< 5	50	50	< 30	400	1,250	1.250
Fusarium sp. 31	< 5	< 5	500	500	< 30	250	1,250	1,250
Fusarium sp. 32	< 5	< 5	< 5	< .5	< 30	< 30	< 30	30
Fusarium sp. 37	< 5	< 5	< 5	< 5	< 30	30	50	100
Fusarium sp. (from asparagus)	< 5	< 5	200	200	< 30	50	200	500
F. bulbigena (from tomato)	< 5	< 5	80	90	< 30	50	150	250
F. apii (from celery)	< 5	< 5	< 5	< 5	< 30	30	50	50
F. hyperoxysporum (from sweet potato)	< 5	150	600	1,000	< 30	500	1,000	2,500

[a] Final pH of all cultures: 6.6–6.8.
[b] Each pellicle was extracted with 50 ml. of 95 per cent ethyl alcohol.

tests for antibiotic activity were performed at one and at two weeks of age on both the culture filtrates and the mycelium. This consisted in draining the pellicles and extracting them in 50 ml. of 95 per cent ethyl alcohol overnight; the antibiotic tests were performed next day on the filtered alcoholic extracts. Previous experience had shown that methyl alcohol and acetone were as effective as ethyl alcohol for the extraction of the antagonistic agent from the mycelium. The antibiotic activity was determined by means of the agar dilution method (21) and the figures express dilution units per 1.0 ml. of test material. Since little or no difference in antibiotic activity was observed between the one- and two-week-old cultures, only one set of figures is given.

By experiment it was determined that 0.33 ml. of alcohol is the largest

amount of this solvent which will allow normal growth of the test organisms; therefore the 30 dilution units was the lowest level of activity detectable for the mycelial extracts.

This experiment yielded the following information:

(1) Of the 18 soil isolates tested, 6 were capable of producing a bacteriostatic principle and this was more pronounced in the glucose-nitrate medium without reinforcements; the addition of 0.5 per cent corn steep liquor completely suppressed the formation of the substance. Glucose-asparagine and glucose-peptone media produced very low antibacterial activity.

(2) The antibiotic substance was more abundant in the mycelium than in the metabolic solution, which shows that it is produced intracellularly and excreted in varying degree into the substrate.

(3) The antagonistic principle was active against the Gram-positive but not against the Gram-negative test organisms used. The usefulness of

TABLE 2.—*Vegetative degeneration and shift in bacteriostatic spectrum of Fusarium hyperoxysporum in glucose-nitrate medium*

Age in days	Mg. residual sugar per ml.	pH	Dilution units per ml. of culture filtrate			
			S. aureus	B. subtilis	B. mycoides	E. coli
0	38.81	6.7	. . ...	. .	. .....	.. ....
3	32.56	6.2	< 5	< 5	< 5	< 5
5	27.61	5.9	< 5	< 5	< 5	< 5
7	22.82	5.7	10	30	30	< 5
10	20.15	6.1	30	65	. . .	.. .
13	17.40	6.0	65	100	·65	20
17	11.00	7.1	100	300	· 350	30
20	5.35	7.2	100	250	250	25

glucose-nitrate medium for the production of antagonistic activity by the *Fusarium* strains having been determined, twenty-one new soil and manure isolates were tested against 4 plant pathogenic strains. The extremely high order of bacteriostatic activity of some of these isolates is shown in table 1.

ANTAGONISTIC MODIFICATION OF ACTIVE STRAINS

Upon transference of the experimental work to Louisville, Kentucky,[5] the active saprophytic and plant pathogenic fusaria suffered three important modifications, namely:

(1) Marked loss of vegetative vigor.

(2) Shift in bacteriostatic spectrum demonstrable by the fact that Gram-negative organisms began to be inhibited in addition to the Gram-positives.

(3) Eventual loss of the antagonistic power.

Table 2 illustrates the new behavior of *Fusarium hyperoxysporum* before its bacteriostatic ability was completely lost. The loss of vigor was evident by the slow growth made and by the fact that thick and convoluted pellicles

were no longer formed. Besides these visible characters, the cultures failed to utilize the sugar completely and the residual pH of the medium did not rise. The alcoholic extracts of the scant mycelium obtained showed very low antagonistic activity.

VEGETATIVE REACTIVATION OF STRAINS

It was soon found that the poor growth made by the strains was due to an insufficiency of zinc in the tap water. It is well to recall in this connection that the formula of the glucose-nitrate (Czapek-Dox) medium calls for 10 p.p.m. ferrous sulphate but for no added zinc. When this element was added in Louisville in the proportion of 10 mg. of zinc sulphate per liter of medium, vigorous growth resulted in one week, the sugar was completely and rapidly utilized, and the pH of the metabolic solution rose to 8.5 or more. Where zinc was added the resulting pellicles were thicker, raised, and wrinkled, while the remaining treatments produced thinner and lighter pads with no convolutions.

This restoration of vegetative vigor was not accompanied, however, by the expected recovery of the antagonistic power. The beneficial effect of zinc on growth is in line with the findings of other investigators (9, 10, 11, 17) for molds of the genera *Rhizopus, Aspergillus,* and *Penicillium.*

ANTAGONISTIC REACTIVATION OF FUSARIUM HYPEROXYSPORUM.
TWO-MEMBERED CULTIVATION WITH BACTERIA
AND SPORE SELECTION

It has been shown elsewhere (22) that the enrichment of normal soils with live and washed *Escherichia coli* cells brings about a decided stimulation in the number of fusaria in the soil, and it is well to mention that advantage was taken of this phenomenon for the isolation of saprophytic strains in the preliminary phase of the present work. None of the isolates, however, had been able to inhibit the growth of *E. coli,* although this bacterial species was inhibited later and for the first time in Louisville by a plant pathogenic *Fusarium.* In both cases, nevertheless, antagonistic capacity was eventually lost.

In an attempt to study in more detail this stimulative effect of bacterial enrichment upon fusaria and its possible role upon their antagonistic behavior, two-membered cultures of *Fusarium hyperoxysporum* with *Escherichia coli* and with *Bacillus mycoides* were prepared in sterilized soil accompanied by a pure culture of the fungus in soil alone. The cultures were incubated at 28° C. and kept moist by periodic irrigations with sterile water. Microscopic examination and periodic platings into glucose-peptone and nutrient agar showed that the organisms had been firmly established in the soil mass. Constant watch was maintained on their purity.

After one month of incubation a series of platings was made from the pure and the two-membered cultures on acidified glucose-nitrate agar, and individual colonies were transferred into duplicate slants of the same

medium. Parallel platings were made from the regular stock culture, which, when propagated directly by means of mass transfer, consistently yielded growth with very little or with no antagonistic capacity. This stock culture had been maintained in soil-extract agar (12) because it had been previously found that this substrate produced growth with more raised mycelium. Five colonies from each substrate were propagated using one member of each duplicate.

The propagation was made by the usual technique of suspending the growth from the agar slants in a small volume of sterile water or saline, and inoculating one ml. of the suspensions in duplicate into 50-ml. volumes of glucose-nitrate broth of pH 7. This time the medium was reinforced with 10 p.p.m. of zinc sulphate to ensure vigorous growth. The 40 cultures were incubated at 28° C. and tested at 1 and at 2 weeks of age for antibiotic activity against *Bacillus mycoides* and *Escherichia coli* by the agar dilution method. One member of each pair was used each time and both the metabolic solutions and the alcoholic extracts of the mycelium were tested. Since

TABLE 3.—*Antibiotic activity of colonies of Fusarium hyperoxysporum obtained by plating 2-membered and pure cultures from different substrates*

Substrate	*B. mycoides* dilution units per ml. of alcoholic extract of mycelium[a]				
Soil	50	< 30	< 30	< 30	50
Soil and *E. coli*	300	< 30	650	300	< 30
Soil and *B. mycoides*	< 30	< 30	100	< 30	150
Soil extract agar	< 30	400	300	300	< 30

[a] Each pellicle was produced in 50 ml. of medium in a 250-ml. Erlenmeyer flask and was extracted with 50 ml. of 95 per cent ethyl alcohol.

the antibiotic level of the solutions was almost negligible, only the results of the mycelial extracts are recorded in table 3. The antagonistic titer remained unchanged after the first week of incubation. No inhibition could be recorded against *E. coli* in any of the cultures.

The most striking result of this experiment was the manifest ability of spores proceeding from the degenerated stock culture to produce antagonistic pellicles. This was certainly a very unexpected result in view of the consecutive number of inactive and nearly inactive cultures previously obtained from it by mass transfer.

In regard to the very important point of the influence of *Escherichia coli* upon the antagonistic behavior of the fungus, the results were negative. This agrees with the results obtained by Waksman and Schatz (22) with normal soil cultures in which the increased number of fusaria obtained by enrichment with *E. coli* failed to inhibit this Gram-negative organism although antagonizing considerably the Gram-positives.

The spores previously harvested from the highest testing pellicle of table 3 were plated out and 20 of the resulting colonies propagated and tested. The process was repeated twice more, the previously harvested spores of

the highest testing pellicle of each generation always being plated.   The results of this work can be summarized thus:

(1) No increase above the 650 *Bacillus mycoides* dilution units level was obtained.

(2) Inactive pellicles were eliminated by selection.

(3) The general level of activity was improved in the first two generations but receded again in the third.

(4) Not one culture inhibited *Escherichia coli*.

EFFECT OF ZINC ON THE BACTERIOSTATIC SPECTRUM OF FUSARIUM HYPEROXYSPORUM

It has been shown that when the antagonistic reactivation of the fungus was accomplished in Louisville through vegetative segregation, the antibacterial spectrum obtained conformed, not to the one observed there before the mold lost its activity, but to the original pattern of inhibition previously obtained in New Brunswick.   Moreover, the fact that the reactivation had been registered in glucose-nitrate medium reinforced with zinc (in order to ensure vigorous growth) strongly suggested that perhaps the absence of this element might have been responsible for the shift in antibacterial spectrum already noted.

This possibility was investigated upon resumption of the work in New Brunswick.   The study consisted in observing again the antibacterial behavior of *Fusarium hyperoxysporum* grown in glucose-nitrate medium with different levels of zinc and other metals and using a wider range of test bacteria.   In order to refine the conditions, the water used for the preparation of the medium was freed from trace elements by the Steinberg technique (18), which consists in thoroughly mixing the medium with 2.5 per cent calcium carbonate followed by heating under steam pressure and filtering. All glassware was rinsed with this purified water.   The chemicals used in the preparation of the media were of Merck Reagent grade, except the glucose, for which commercial ''cerelose'' was used.   The basal medium consisted of the original Czapek-Dox formula, which includes 10 p.p.m. added ferrous sulphate but no zinc.

It was possible to show that when antagonistically active cultures of *Fusarium hyperoxysporum* are grown in glucose-nitrate medium prepared with ordinary tap water or with purified distilled water reinforced with zinc, the antibiotic principle produced is active against Gram-positive bacteria only.   When the medium is prepared with distilled water purified by the Steinberg method, however, the antibiotic substance produced can inhibit both the Gram-positive and the Gram-negative organisms.

Likewise, under conditions of zinc deficiency, the active principle is readily excreted into the metabolic solution in contrast with the ordinary zinc-satisfied cultures in which the activity remains tied up in the mycelium. It was likewise observed that the zinc-deficient growth produced higher levels of activity in submerged cultivation obtained with a rotary shaking

machine. These cultures produced scant mycelium, failed to consume all the sugar, and their hydrogen ion concentration remained close to neutrality, which was precisely the behavior observed in Louisville before the medium was reinforced with zinc. Table 4 illustrates a typical experiment. Further information obtained in this study can be summarized thus:

(1) The zinc effect takes place in the presence as well as in the absence of iron.

(2) Manganese and copper do not exert the slightest effect on the behavior of the fungus.

(3) Individual spore isolates, although showing marked quantitative variability, behave in exactly the same way qualitatively toward the metal.

TABLE 4.—*Effect of zinc upon the bacteriostatic spectrum of Fusarium hyperoxysporum*

Test bacteria	Dilution units per ml. after 14 days of incubation	
	Stationary cultures[a]	Shaken cultures[b]
	Alcoholic extract of mycelium; medium with 10 p. p. m. zinc sulphate added	Metabolic solutions of zinc-free medium
Bacillus mycoides	500	90
B. subtilis	500	150
Staphylococcus aureus	200	35
Escherichia coli	< 30	40
Shigella paradysenteriae	< 30	150
Salmonella pullorum	< 50	90
Proteus vulgaris	< 30	50

[a] Produced in 50 ml. of medium in 250-ml. Erlenmeyer flasks.
[b] Produced in 100 ml. of medium in 250-ml. Erlenmeyer flasks.

Because of their difference in antibacterial spectrum and in mode and characteristics of formation, the two antibiotic substances produced by *Fusarium hyperoxysporum* were regarded as distinct from one another and tentatively were designated as A and B.

Thus fraction A stands for the substance produced in the presence of zinc, active mainly against the Gram-positive bacteria, and obtainable only from the mycelium of the fungus in its present level of antagonistic activity.

Fraction B designates the substance produced in the absence of zinc, active against both Gram-positive and Gram-negative bacteria and obtainable from the metabolic solution.

EXTRACTION OF FRACTION A FROM MYCELIUM

The fungus was cultivated in 10 liters of glucose-nitrate medium prepared with tap water and reinforced with 5 p.p.m. of zinc sulphate to ensure against any deficiency of this element. After two weeks' incubation at 28° C. in stationary culture, the pellicles were separated from the medium, squeezed through cheesecloth, and extracted with three consecutive half-

liter volumes of 95 per cent ethyl alcohol for 24 hours each time. pooling and filtering, a volume of 1,675 ml. of a golden brown extract was obtained.

Previous experience had demonstrated that this alcoholic extract would not yield any of its antibacterial activity to Norit A, a powdered charcoal adsorbent, when undiluted, but that, upon dilution with an equal volume of distilled water, more than 90 per cent of the activity could be adsorbed in 1 per cent Norit based on the final volume. The antibiotic test made on the final 3,350 ml. of dilute extract gave the following values in dilution units per ml.:

Bacillus mycoides	750	*Shigella paradysenteriae*	< 30
Staphylococcus aureus	300	*Salmonella pullorum*	< 50
Escherichia coli	< 30	*Proteus vulgaris*	< 30

The liquid was then treated with 1 per cent Norit A with mechanical stirring for one-half hour; the antibiotic test performed with the filtrate showed 25 dilution units for *Bacillus mycoides* and no detectable action for *Staphylococcus aureus*.

Previous experience had shown that, once the active principle is adsorbed in the Norit, it cannot be eluted with neutral or acid alcohol but passes readily into alkaline alcohol prepared by mixing 11 parts of 95 per cent ethyl alcohol with 4 parts of distilled water alkalinized with 2 per cent NaOH based on the final volume. Because of this experience the Norit was eluted three times in succession with 2 per cent alkaline alcohol at room temperature for two hours each time and with frequent shaking. Pooling of the eluates gave a volume of 845 ml. with a titer of 1,200 *Bacillus mycoides* dilution units per ml. after neutralization with concentrated HCl. This is equivalent to 40 per cent recovery for this test organism.

After concentration of the eluates to 200 ml. under diminished pressure, the resulting cloudy suspension was slowly added with constant stirring to 2 liters of acetone, the salts were filtered off, and the acetone was removed by distillation. After it was ascertained that more than 90 per cent of the antibiotic activity was in the organic fraction, this was carefully collected, made up to 200 ml. with distilled water, and extracted twice with four volumes of diethyl ether with vigorous shaking. Evaporation of the ether layers produced 500 mg. of a brown, viscous residue with a pungent odor and which could not be dried under vacuum. The antibiotic spectrum obtained with the alcoholic solution of this crude preparation is given in table 5. It can be seen that this spectrum bears no resemblance to the antibacterial spectra of penicillin, chaetomin, or fumigacin (19).

ISOLATION OF FRACTION B FROM METABOLIC SOLUTION

Ten liters of glucose-nitrate medium were prepared with distilled water purified by the Steinberg technique already described and inoculated with spores of *Fusarium hyperoxysporum*. Because the incubation was done in

stationary culture the antibacterial titer of the metabolic solution remained very low even after 20 days at 28° C. The growth of the fungus was very poor in this zincless medium and the pH did not rise above 7.2 After separation from the mycelium and filtration, 8.0 liters of metabolic residue were obtained. The antibiotic strength of this solution, measured in dilution units per ml. was as follows:

Bacillus mycoides	25	*Proteus vulgaris*	5
Escherichia coli	5	*Shigella paradysenteriae*	20

The active filtrate was now treated with 80 grams of Darco activated carbon (grade G–60) by mixing at room temperature for one hour with

TABLE 5.—*Antibiotic spectra of Fusarium fractions A and B*

Test organism	Dilution units per 1.0 mg. dry weight of final alcoholic solution	
	Fraction A	Fraction B
Staphylococcus aureus (ATCC 6538)	117	20
Sarcina lutea No. 14	290	10
Micrococcus lysodeikticus No. 19	190	20
Bacillus cereus No. 8	784	40
B. subtilis (ATCC 6633)	686	40
B. mycoides (ATCC 6462)	585	35
B. megatherium No. 10	190	35
B. circulans	190	35
Mycobacterium phlei No. 23	137	15
M. tuberculosis var. *hominis* (ATCC 607)	30	5
Escherichia coli No. 4	< 10	15
Proteus vulgaris No. 73	30	15
Proteus vulgaris No. 74	30	15
Serratia marcescens (ATCC 989)	< 10	5
Salmonella pullorum (PCI No. 904)	< 10	35
Shigella paradysenteriae ("V"9380–ATCC)	< 10	35
Aerobacter aerogenes No. 66	< 10	< 5
Pseudomonas fluorescens No. 68	< 10	< 5
Candida albicans No. 173	15	15
Trichophyton mentagrophytes No. 171	30	10
Trichoderma sp. No. 102	15	< 5
Fusarium hyperoxysporum Woll.	< 10	< 5

mechanical stirring. After overnight standing in the refrigerator the carbon was filtered off and dried at 55° C. overnight followed by 24 hours at room temperature. The dried adsorbent was then treated with 400 ml. of diethyl ether for 24 hours followed by elution with 800 ml. of 95 per cent ethyl alcohol overnight at room temperature. This preliminary treatment with ether was adopted because previous experience had shown that it facilitated the removal of the antagonistic substance by the alcohol. The alcohol eluate was then filtered and evaporated under diminished pressure to a very small volume followed by overnight drying at 55° C. in the dry oven. The complete evaporation of the alcohol and water resulted in a light brown and transparent brittle residue which was then redissolved in 50 ml. of warm 95

per cent alcohol. Treatment of this alcoholic concentrate with ten volumes of cold distilled water produced precipitation of a white crystalline fraction. Two more precipitations were obtained by evaporating the mother liquor under diminished pressure, redissolving the residue in alcohol, and treating again with water; 1.7 grams of dried crystalline preparation was obtained from 8 liters of metabolic solution. The antibiotic spectrum of this substance is summarized in table 5; it bears some resemblance to the spectrum of penicillic acid (19).

BEHAVIOR OF FRACTIONS A AND B "IN VIVO"[6]

Toxicity and efficacy tests conducted with samples of the two substances showed that fraction A is nontoxic when administered subcutaneously in concentrations of 125 *Staphylococcus aureus* dilution units per mouse. At this concentration, however, it failed to protect the animals against infection with *S. aureus SM*. This suggests inactivation or destruction of the substance in the animal organism.

The *in vivo* work with fraction B was complicated by the low antibiotic potency of the crystalline preparation, which required the administration of 5.0 mg. per mouse in order to bring the antibiotic unitage to the minimum level necessary for the efficacy tests. This dose was found to be toxic in subcutaneous injection. The efficacy tests showed that when doses just below the toxic level were administered the substance failed to protect the experimental animals against *Salmonella schottmülleri*. It might be possible that further purification of this antibiotic can increase its potency to a point at which protection would be feasible with doses below the toxic level.

SUMMARY

When several saprophytic and plant pathogenic fusaria were propagated in simple synthetic media like glucose-nitrate (Czapek-Dox) broth, they readily produced antibiotic substances. This property was quickly lost, however, when the stock cultures were perpetuated by mass transfer but was regained through the propagation of individual spores.

Further work with *Fusarium hyperoxysporum* demonstrated that the antagonistic potency of this organism against the Gram-positive bacteria could be enhanced to some degree by two-membered cultivation with *Escherichia coli* in sterile soil. This association, however, did not result in the inhibition of *E. coli* by the fungus.

The qualitative antagonistic behavior of *Fusarium hyperoxysporum* was found to be governed by the quantity of zinc present in the culture medium. In the presence of this element the fungus produces an antibiotic active mainly against Gram-positive bacteria; in its absence both the Gram-positive and Gram-negative organisms are inhibited.

Both antibiotic principles were isolated from mass cultures of *Fu-*

sarium hyperoxysporum and designated tentatively as A and B.   Further chemical work is needed to identify and characterize these substances.   Fraction A proved to be nontoxic to experimental animals but failed to convey protection against infection with *Staphylococcus aureus SM*.   Fraction B was toxic in doses of 5.0 mg. per mouse, and when administered in amounts below the toxic level it did not protect the animals against *Salmonella schottmülleri*.

In its mode of formation and in antibacterial spectrum, fraction A is distinct from penicillin, chaetomin, and fumigacin, while fraction B bears some resemblance to penicillic acid.   Further work is needed to elucidate the true nature of these active principles.

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PHYTOPATHOLOGICAL NOTES

Seed Treatment with Mercury Dusts Injurious to Corn with Mechanical Injuries Near Embryo.—Severe injury following seed treatment of corn with mercury dusts applied at normally safe dosages was observed in a field experiment at Madison, Wisconsin, in 1946. Semesan Jr. (1 per cent ethyl mercury phosphate), and Du Pont 1451-D (1.25 per cent ethyl mercury p-toluene sulfonanilide), the two mercury disinfectants included in the experiment with mechanically injured seed, both caused typical mercury injury to nearly all germinating seedlings. The kernels, which had been injured by rupturing the pericarp with a scalpel along the edges of the embryo, were treated on April 13 and planted in the field on May 28. The treated seeds were kept in paper packets in the laboratory, at room temperatures, during the 46-day storage period.

Seeds similarly injured but planted the day they were dusted (April 13) resulted in no mercury damage and responded favorably to the seed treatments with large increases in stands of healthy seedlings over those of the untreated controls. Likewise, seed that had been subjected to crown and tip-cap injuries showed no evidence of mercury injury in either the April or May plantings.

Proof that the mercury injury resulted from storage of the treated seed for an appreciable period before planting, rather than from differences in soil environment when the April and May plantings were made, was established in a subsequent greenhouse experiment. Seed from a mixture of several commercial hybrids was subjected to pericarp injuries as described above and then divided into two lots. One lot was treated with Semesan Jr. immediately after the kernels were injured, and the other 76 days later when both lots were planted in a greenhouse bench, in twelve 25-kernel replications. Nearly every seedling from the seed stored for 76 days before planting developed the stunted and swollen condition typical of mercury injury in contrast with normal seedlings from the seed treated at the time it was planted (Fig. 1). The time of storage required before mercury injury develops in seed having pericarp injuries near the embryo obviously was not determined in these experiments.

The information obtained probably is of no great practical importance but it discloses a fault of mercury dusts not mentioned heretofore in the literature on corn seed treatment.—P. E. HOPPE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

Sclerotium delphinii Welch on Scilla.[1]—A disease of scilla (*Scilla sibirica*) was observed early in May, 1940, in a commercial planting located

FIG. 1.   A. Corn seedlings showing the range of mercury injury (stunting and swollen tissues) resulting from Semesan Jr. treatment of wounded seed stored 76 days before planting; B. Normal seedlings from the same lot of wounded seed planted the day it was dusted.

near Tacoma, Washington. The greater part of the planting (Fig. 1, a) was completely destroyed by the disease, and upon lifting the bulbs, over 90 per cent of the remaining plants were found to be infected.

Symptoms on the above-ground parts were (1) the yellowing and drying up of the leaves, beginning at the tips, and (2) wilting of the blossoms, with the flower stalks remaining erect until the fungus had advanced well above the ground-level.
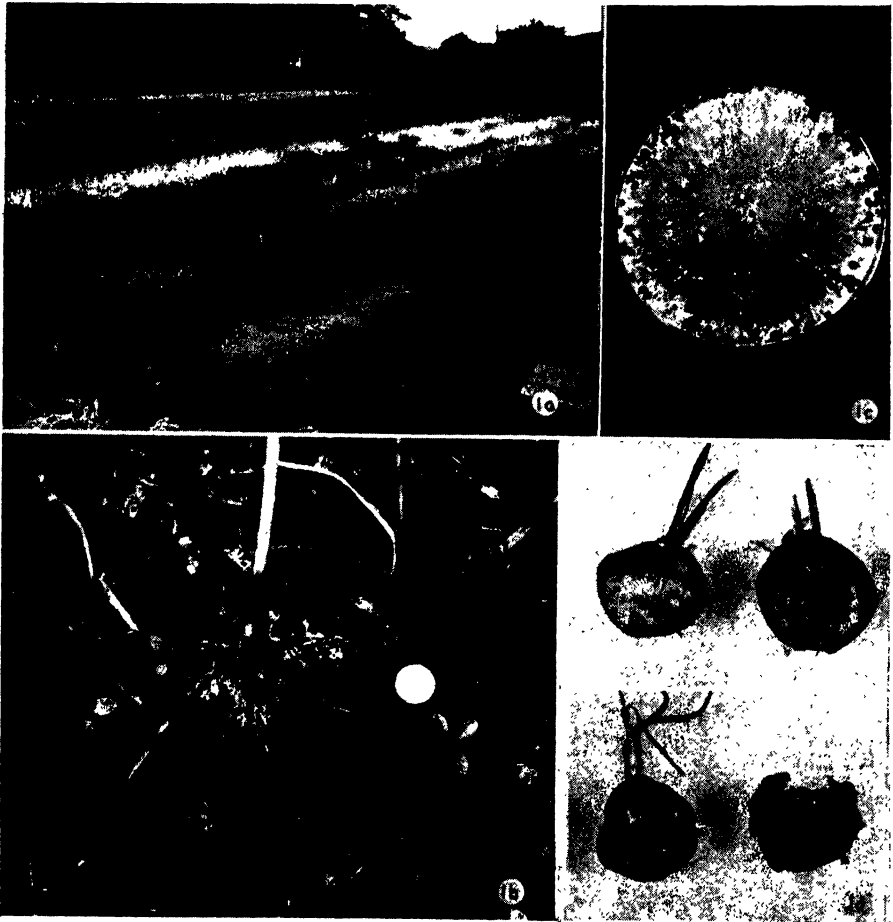
FIG. 1.   a. Scilla planting showing destruction of plants by *Sclerotium delphinii*. b. Diseased plant showing mycelial strands on the surface of the soil.   c. Plate culture of *Sclerotium delphinii*.   d. Scilla bulbs showing various degrees of decay caused by *Sclerotium delphinii*.

Infection apparently started on the bulbs with the fungus advancing from them into the above-ground parts of the plant. Compact strands of mycelium spread over the surface of the soil from the decayed leaves and flower stalks (Fig. 1, b). In the badly infected areas of the beds, fragments of decayed leaves, covered with compact mats of mycelial growth, were

present on the surface of the soil. Reddish-brown to dark brown sclerotia were scattered on the surface and in the soil in the area immediately surrounding the diseased plants.

Infections on the bulbs ranged from small, slightly depressed darkened areas to complete decay. Small dark reddish-brown sclerotia were found in the decayed tissue of the bulbs.

Platings were made on potato-dextrose agar from bulbs showing various stages of infection, from the mycelial mats and strands on the surface of the soil, from sclerotia in the soil, and from infected leaves and flower stalks. White nonsporulating mycelial colonies producing reddish-brown sclerotia in 10 to 14 days (Fig. 1, c) developed from the plantings.

A giant oat-culture of the fungus was mixed with sterilized silt soil in a greenhouse bench and 24 apparently healthy bulbs were planted 6 inches apart in the inoculated soil. Plants from 6 bulbs emerged from the soil, only one of which survived to produce a flower stalk. The remaining 18 bulbs decayed before plant emergence. The same fungus was recovered from all of the infected bulbs. Bulbs planted in non-inoculated sterilized soil developed normally, free from the disease.

One-hundred apparently healthy bulbs were planted in inoculated soil in the field. Four plants failed to emerge from the soil. Examination of these bulbs showed that the bulbs started to decay before the root systems became established. Twenty-two per cent of the plants reached full bloom. Examination of the bulbs five months later revealed lesions varying from 1/16 of an inch in diameter to complete decay of the bulb (Fig. 1, d). Five bulbs showed no apparent infection.

Cultures and partially decayed bulbs were sent to Dr. Donald M. Coe, formerly of the Department of Plant Pathology, State College of Washington, Pullman, Washington, who identified the causal organism as *Sclerotium delphinii* Welch. He stated that the sclerotia of the causal fungus varied only slightly as to size and color from the cultures isolated from Delphinium. However, "it is possible that this may be an additional strain of the fungus."[2]—GLENN A. HUBER and C. J. GOULD, Western Washington Experiment Station, Puyallup, Washington.

Antagonistic Activity of a Species of Actinomyces against Ceratostomella ulmi in vitro.[1]—Increasing knowledge of antibiotics during recent years and recognition of their proved therapeutic value in combating human diseases have stimulated interest in the use of these products for control of plant diseases. Lack of adequate preventive or curative measures for the Dutch elm disease warrants investigation of such materials. This report describes

the antagonistic effect of a species of *Actinomyces* on *Ceratostomella ulmi*, the causal fungus of the disease.

Working with a strain of *Ceratostomella ulmi*, Waksman and Bugie[2] reported in 1943 that the antibiotics actinomycin and clavacin exhibited high activity in laboratory tests against this fungus.

Preliminary tests have indicated that the species of *Actinomyces* used in the present work is distinctly different from *A. antibioticus*, the source of the antibiotic actinomycin. The isolate was originally obtained from C. M. Haenseler who observed it as a contaminant on agar plates.

The isolate of *Ceratostomella ulmi* used in these tests is known as a
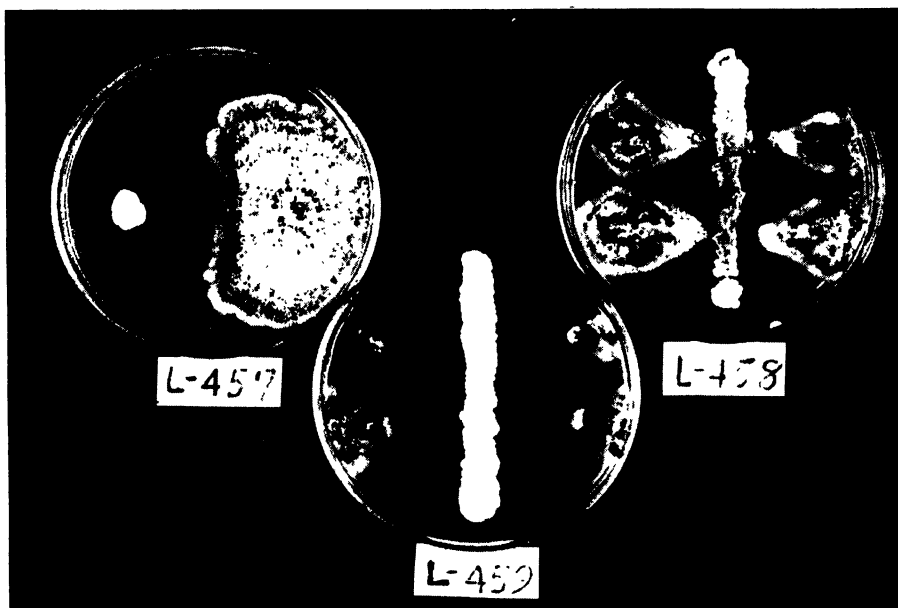
FIG. 1. Antagonistic effect of a species of *Actinomyces* on *Ceratostomella ulmi*. Plates L–457 and L–458: both organisms streaked the same day. Plate L–459: *C. ulmi* streaked 3 days after the *Actinomyces* streak.

"black line strain" and was supplied by the Department of Plant Pathology, Cornell University, under the label, Tyler No. T114a 2aR. It is one of the most virulent strains of the Dutch elm disease fungus thus far isolated from infected trees. Its virulence was tested on 10 healthy trees in May, 1946 before the *in vitro* tests were started. Typical symptoms of the disease appeared on all trees in 8 to 14 days. The organism was recovered in all cases at some distance from the point of inoculation.

Two *in vitro* methods, the agar streak[3] and the two-point inoculation, were used to test the antagonistic action of the *Actinomyces sp.* to *Ceratostomella ulmi*.

[2] Waksman, S. A., and E. Bugie. Action of antibiotic substances upon *Ceratostomella ulmi*. Proc. Soc. Exp. Biol. and Med. 54: 79–82. 1943.

[3] Waksman, S. A., and H. C. Reilly. Agar-streak method for assaying antibiotic substances. Ind. and Eng. Chem. 17: 556. 1945.

In the first method, a long streak of the *Actinomyces sp.* was made across the diameter of a Petri plate containing potato-dextrose agar. Four short streaks (two on each side and at right angles to the long streak) were made with the *Ceratostomella ulmi*. In some plates all streaks were made on the same day; in others, the *C. ulmi* was streaked 1 to 3 days after the *Actinomyces*.

The second method involved the planting of each organism at opposite sides of a poured plate to allow the organisms to grow toward each other. An advantage of this method is that both organisms can be started simultaneously yet sufficient time is provided for the *Actinomyces sp.* to produce its antagonistic substance.

All plates were incubated at 23° to 25° C. and examined periodically.

When the streaks of both organisms were made on the same day, distinct inhibition of *Ceratostomella ulmi* by the *Actinomyces sp.* was evident 1 to 2 days following its streaking. *C. ulmi* did not grow within 20 to 24 mm. of the *Actinomyces* (Fig. 1, L–459).

When the two organisms were planted at opposite sides of the plate, the first indication of the antagonism was observed within 6 days (Fig. 1, L–457). That the inhibitory action began early is indicated by the flattening of the *Ceratostomella ulmi* growth rings, which normally are circular. The zone of inhibition between the *Actinomyces sp.* and *C. ulmi* measured more than 20 mm., and in some cases more than 25 mm. The strong inhibitory activity against *C. ulmi* was retained for 2 months, by which time the medium had dried completely.

The results herein reported both for the streak method and the two-point inoculation method have been repeated more than 10 times with the same consistent results. The antagonistic activity of the *Actinomyces sp.* is entirely independent of pigment formation by the organism.

Efforts to extract the active material from cultures of the *Actinomyces sp.* grown on solid, semi-solid, and liquid media have not succeeded to date. Occasional filtrates and crude extracts with ether show some activity against *Ceratostomella ulmi*, as measured by the cup method technique.—MICHAEL SZKOLNIK, Department of Plant Pathology, Rutgers University, New Brunswick, New Jersey.

THE USE OF VAPOR–HEAT AS A PRACTICAL MEANS
OF DISINFECTING SEEDS[1, 2, 3]

P. W. Miller[4] and F. P. McWhorter[5]

Since Pasteur's time numerous studies of the factors that determine the ability of heat to kill microorganisms have been recorded in various bacteriological and physiological texts. These indicate that the higher the water content of the medium in which the organism is contained the lower the temperature and the shorter the exposure required for its death. These observations may have led early phytopathologists, beginning apparently with Jensen in 1887 (2), to utilize hot water to kill pathogens within seeds. Since 1887 specific hot-water treatments for various pathogens and seeds have been developed and recommended. These have not become popular, however, because (a) the treatments require critical time and temperature control, (b) the seeds must be quickly cooled and dried, (c) enormous space is required for drying large amounts of seed, and (d) injury frequently accompanies treatment even when all precautions are observed.

Coincident with the development of the hot-water method of seed disinfection dry heated air was utilized by certain investigators to rid seeds of microorganisms. Critical summaries of these investigations by Atanasoff and Johnson (1) in 1920 and by Lehman (5) in 1925 indicate that temperatures of 95° C. to 100° C. for 1 to 3 days are required for disinfecting seeds with dry heat. Moreover, to avoid injury at the high temperatures, the seeds must be thoroughly dried before beginning the treatment. The long exposures, the possibility of injury, and the contingent fire hazard have discouraged the use of dry heat treatment by commercial seedsmen.

The utility of disinfecting seeds with an intermediate form of heat called "vapor-heat" was investigated by us during 1941 to 1946 as part of the coordinated war-time research program of the American Phytopathological

Society. The application of heat in a manner intermediate between heated water and heated dry air is accomplished by introducing steam into a current of air with a mechanical arrangement adjusted to maintain a desired temperature and proportionate moisture content. The term vapor-heat for such steam-air mixtures was apparently first suggested by Hawkins and introduced by Latta (3-) in connection with the disinfestation of narcissus bulbs.

A preliminary report of our investigations has already been made (6). This more detailed account is presented to show the practical value and limitations of vapor-heat for seed treatment. The method avoids extreme wetting and drying, thus eliminating many of the objections mentioned above. The principle, if applied in suitable automatic machinery, seems well adapted for large-scale operations by seedsmen.

THE VAPOR-HEAT MACHINE AND METHODS

The equipment used in these tests is essentially the same as devised and described by Latta (3, 4). Steam at 10 to 20 pounds pressure is released through a series of small openings in a U-shaped pipe and mixed or "vaporized" with air in a sheet-metal conditioning box approximately 5 feet long, 3 feet wide, and 3 feet high. From the conditioning box, the vapor passes through a metal conduit into a double-walled, wooden treating room approximately $5 \times 5 \times 4$ feet (Fig. 1). The hot vapor enters the treating room through a square opening in the center of the ceiling and is distributed by a perforated baffle plate suspended about 6 inches below the opening. The perforated plate allows a portion of the mixture to flow downward into the center of the room. The vapor is subsequently withdrawn from the lower part of the room and circulated again through the conditioning box and reintroduced through the ceiling by means of a fan placed in the circulation "stream." The temperature of the mixture is held constant to within $\pm 2°$ F. by Mercoid thermostatic control equipment which operates a steam valve introducing more steam into the mixture as needed to maintain this required temperature.

The use of vapor-heat for seed disinfection being new, a large number of temperature combinations on different seeds and pathogens were tested before a somewhat standardized procedure was attained. Later, we always used low temperature approach periods followed by short higher temperature exposures. Since these temperatures extended from 110° F. to 165° F. and over 800 operations of the vapor machine were involved it is possible to include only a small portion of the records in the accompanying tables. Temperatures and combinations which later experience proved too low have been largely omitted.

The seeds to be treated were placed in small trays, measuring $11 \times 11 \times 3$ cm. with 32-mesh wire screen bottoms and holding approximately 35 gm. of table beet seed and 75 gm. of cabbage seed. The seeds were transferred aseptically to sterile paper sacks after treatment in the vapor-heat machine, and were subsequently plated out on potato-dextrose agar to determine the

viability of the microorganisms present on or within the seeds.   At the same time comparable untreated seeds were plated out as controls.   Seeds in germination and infection studies were handled in a similar manner.

The viability of seed by laboratory methods was determined as the average germination of two samples of 100 seeds each.

The germination by rate of emergence was determined as two to four replicates of 100 seeds, or seed-balls, each, planted in pasteurized soil.   The
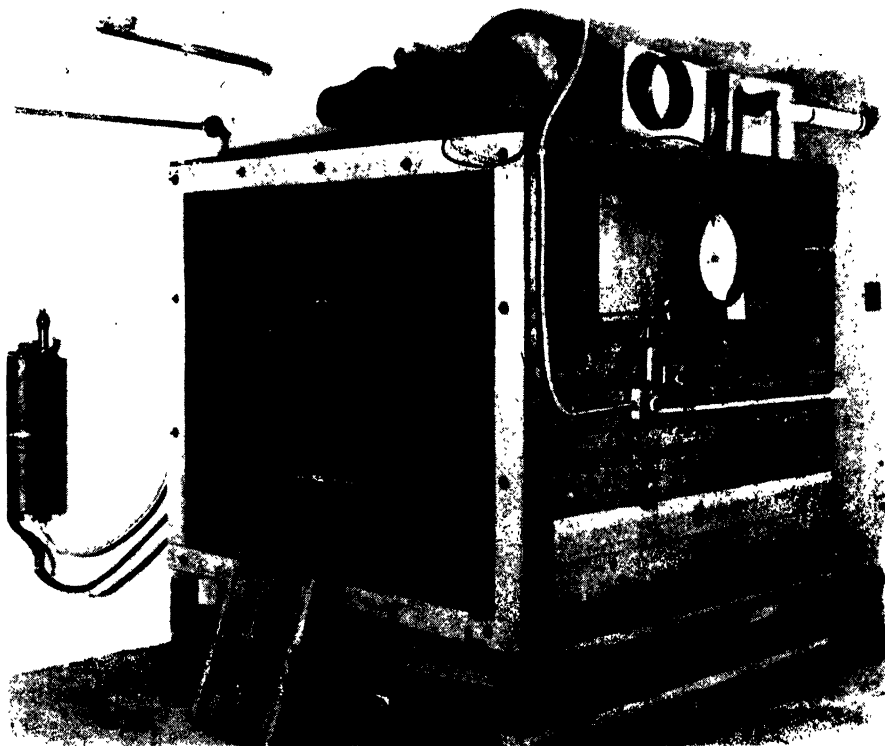
FIG. 1.   The vapor-heat machine.

emergence of the seed was recorded as a percentage of the seed sown and the mean emergence period in days was thus determined.

RESULTS

Efficacy of vapor-heat to disinfest seeds contaminated with molds.—Preliminary trials of disinfestation with vapor-heat were made on naturally infested seeds of beet, carrot, cabbage, cauliflower, brussels sprouts, onion, pea, snap bean, radish, red clover, bentgrass, Chewings fescue, and perennial ryegrass.   The seeds were randomly infested with species of *Macrosporium, Stemphyllium, Fusarium, Aspergillus,* and *Penicillium.*

Trials included 53 time-temperature variations with or without approach or warm-up periods.   These ranged from 117° F. for 30 minutes to 160° F.

for 40 minutes.[6] The results were erratic due to great variation in the fungus flora of different seeds and to the fact that *Penicillium* appeared in cultures representing temperature ranges where control of the other fungi was consistent. We were never certain whether the unpredictable occurrence of *Penicillium* in cultures of treated seeds was attributable to chance recontamination during the transfer of the seeds from the treating room to agar plates, or to resistance of the fungus on the seeds to vapor-heat. Nineteen per cent of 835 seeds plated as controls proved uncontaminated with fungi, thus adding further experimental error. It was concluded that vapor-heat exposures of 150° F. for 30 minutes or combination exposures of 130° F. for 15 minutes followed by 140° F. for 45 minutes will destroy most contaminating molds. The use of an approach period of 130° F. for 15 minutes com-

TABLE 1.—*The effect of vapor-heat on certain fungi*

Vapor-heat treatment	Percentage of seeds inoculated[a] with specified fungi that harbored viable fungus material after vapor-heat treatment for indicated temperatures and exposures		
	Phoma betae	*Botrytis cinerea*	*Fusarium orthoceras*
Not treated	99	98	100
125° F., 5 min.	40	20	. . . .
125° F., 10 min.	20	0	
125° F., 20 min.	0	0	. ...
130° F., 5 min.	10	10	. ....
130° F., 20 min.	0	0	0
135° F., 5 min.	30	0	0
135° F., 10 min.	20	0	0
135° F., 20 min.	0	0	0
140° F., 5 min.	0	0	0
140° F., 10 min.	0	0	0
140° F., 15 min.	0	0	0

    [a] Table beet seed-balls were first autoclaved to kill the natural microflora and then soaked in spore suspensions of the respective fungi.

bined with a short treatment of 140° F. to 145° F. proved consistently better than single treatments of 150° F. for proportional periods. There was some indication that smooth seeds, such as cabbage, were more easily disinfected than such rough seeds as beet "seed-balls."

Ability of vapor-heat to kill specific fungi on and in beet "seed-balls."— The vapor-heat "death points" for *Phoma betae* (Oud.) Frank, *Botrytis cinerea* Pers., and *Fusarium orthoceras* Ap. et Wr. were obtained by first soaking autoclaved beet seed-balls in suspensions of the spores of the respective fungi and allowing the fungi to grow into the coats of the fruits.

The data given in table 1 show that *Fusarium orthoceras* was especially susceptible to vapor-heat and that exposure to a temperature of 140° F. for 20 minutes should kill any of the three fungi when in a vegetative condition.

    [6] These data are too voluminous to warrant tabulation and are therefore merely summarized in this paragraph. Also, the many variables kept this preliminary study from being precise.

Vapor-heat "death points" of sclerotia of Sclerotinia.—The very short exposures and relative low temperatures required to inactivate sclerotia of *Sclerotinia minor* Jagger and *Sclerotinia sclerotiorum* (Lib.) De Bary from cabbage are of special interest. Table 2 shows that sclerotia of *S. minor* were killed by vapor-heat treatment at only 130° F. for 15 minutes. This is at least 15 degrees F. and 45 minutes' exposure below the analogous thermal death-point of cabbage seed (Table 4). Platings of sclerotia that were cut open to eliminate error from delayed germination showed that all sclerotia of both species were killed by treatments of 135° F. for 30 minutes. Since many sclerotial "pellets" sometimes remain among cabbage seed after mechanical cleaning, vapor-heat may prove advantageous for disinfesting cabbage seed.

Specific control of seed-borne diseases.—The results of limited studies of controlling specific seed-borne diseases by vapor-heat indicate that this

TABLE 2.—*The effect of vapor-heat on the sclerotia of Sclerotinia from cabbage.*

Organism	Vapor-heat treatment	Number of sclerotia plated	Percentage of sclerotia viable after treatment
Sclerotinia sclerotiorum	Not treated	19	100
Do	130° F., 15 min.	16	31
Do	130° F., 30 min.	6	33
Do	130° F., 45 min.	10	10
Do	130° F., 60 min.	11	27
Do	135° F., 15 min.	6	0
Do	135° F., 30 min.	6	0
Do	135° F., 45 min.	6	0
Sclerotinia minor	Not treated	7	100
Do	130° F., 15 min.	6	0
Do	130° F., 30 min.	6	0
Do	130° F., 45 min.	6	0

method of thermal therapy is practical for certain diseases. However, disinfection of large seeds such as *Phaseolus vulgaris* L., variety White Kidney, infected with *Colletotrichum lindemuthianum* (S. & M.) Bri. & Cav., which penetrates deeply into the seeds is difficult and may be impractical.

The control of *Phoma betae* in naturally infected sugar beet seed-balls by vapor-heat has been demonstrated as recorded in table 7. Moreover, preliminary tests indicate that the vapor-heat treatment of table beet fruits naturally infected with *Phoma betae* may prevent the crown rot disease of table beet roots. This was indicated by tests carried on in 1944–1945 in cooperation with Northrup, King and Company of Minneapolis, Minnesota. In February, 1944, we vapor-heat treated some naturally infected table beet seeds supplied by this company. These seeds were planted by representatives of the company in their trial grounds in Minneapolis along with non-treated seeds from the same source. The roots from both lots were harvested in October, 1944, and stored in a cellar over winter. The treated lot was kept separate from the non-treated lot. When the roots went into storage there was no detectable difference in the two lots. The roots were subse-

quently removed from storage about the middle of April, 1945, and the incidence of "rotted" roots in each lot determined. The untreated roots had about 40 per cent crown-rot while the roots produced from the seeds treated by vapor-heat were practically free from crown-rot, only 9 roots being affected in some seven bags of roots stored.

As was discussed above, the sclerotia of both *Sclerotinia minor* and *S. sclerotiorum* mixed among cabbage seed are easily killed by vapor-heat treatments that are considerably below the maximum treatment that could be used without causing injury to the seed. Treatment of infested seed by vapor-heat is a practical method of preventing this disease from being seed-borne.

TABLE 3.—*The effect of vapor-heat on three bacterial plant pathogens of the genus Xanthomonas*

Host	Bacterial pathogen	Vapor-heat treatment	Number of seeds[a] cultured	Percentage of seeds containing viable bacteria after treatment
Cabbage	*X. campestre*	Not treated	60	58
Do	Do	145° F., 30 min.	20	15
Do	Do	145° F., 60 min.	30	0
Do	Do	145° F., 90 min.	30	0
Do	Do	150° F., 30 min.	10	33
Do	Do	150° F., 60 min.	10	33
Do	Do	150° F., 90 min.	10	0
Snap beans	*X. phaseoli*	Not treated	101	98
Do	Do	147° F., 30 min.	12	58
Do	Do	152° F., 30 min.	19	58
Do	Do	152° F., 60 min.	12	25
Do	Do	157° F., 30 min.	12	33
Do	Do	157° F., 75 min.	12	33
Do	Do	157° F., 90 min.	12	0
Do	Do	162° F., 30 min.	12	17
Do	Do	162° F., 45 min.	12	0
Carrot	*X. carotae*	Not treated	65	97
Do	Do	142° F., 30 min.	14	14
Do	Do	147° F., 30 min.	14	7
Do	Do	152° F., 30 min.	33	15
Do	Do	157° F., 30 min.	17	18

[a] Seeds autoclaved, then soaked in aqueous suspensions of the organism in question and subsequently air-dried under aseptic conditions.

Special cases for application of thermal disinfection by means of vapor-heat.—The vapor-heat method possesses many other possible control potentialities that invite investigation. For example, preliminary tests indicate that it may be used effectively to disinfect plant parts other than seeds, such as onion bulbs, gladioli corms, etc. Many other uses may be found where this easily controlled method of heat treatment may be applied without introducing a difficult problem of drying.

Vapor-heat impractical for control of bacterial diseases.—Bacteria are much more difficult to kill by vapor-heat than are fungi. It has proved impossible to disinfest seeds contaminated with spore-forming bacteria at

temperature ranges herein reported. While non spore-forming bacterial plant pathogens are eventually killed, the temperatures required are too high for most seeds to endure without serious injury. Table 3 shows the control potential of vapor-heat on *Xanthomonas campestre* (Pammel) Dowson, *X. phaseoli* (E. F. Smith) Dowson, and *X. carotae* on cabbage, snap bean, and carrot seeds, respectively. It is apparent from these data that the vapor-heat method alone is impractical for controlling seed-borne bacteria. This observation led to preliminary trials of vapor-heat followed by application of chemical disinfectants. A test by plating technique to compare the relative efficiency of vapor-heat and copper oxide for disinfesting beet fruits showed that a combination of the two attained complete disinfestation. The copper alone did not prevent growth of fungi; the heat alone did not prevent growth of bacteria. Combinations of vapor-heat and chemical seed treatment may lead to better control of seed-borne and soil-borne pathogens.

Tolerance of seeds to vapor-heat.—Seeds of Kentucky Wonder bean, brussels sprouts, Marion Market cabbage, Chantenay carrot, Early Sweet pea, sugar beet, Detroit Dark Red table beet, red clover, bentgrass, Chewings fescue, and perennial ryegrass were tested for tolerance to vapor-heat treatments. The tolerance as recorded in table 4 was determined by (a) standard laboratory seed analysis methods, and (b) the rate of emergence in pasteurized soil. These data show that while the tolerance to vapor-heat of individual kinds of seeds varies considerably, many kinds withstand significantly higher temperatures than are required to kill associated fungi. Thus, table beet seed-balls can evidently endure vapor-heat treatment up to 145° F. for 30 minutes with little, if any, effect on the germination or rate of emergence. This treatment is some 10 degrees F. higher than is required to kill most of the associated fungi.

The wide difference in the vapor-heat tolerance of some of the seeds of closely related plants is noteworthy. For example, a vapor-heat treatment of 145° F. for 45 minutes which has little, if any, effect on the germination of cabbage, may seriously injure seeds of cauliflower and brussels sprouts. Similar variations among varieties or lots of the same species may be anticipated (Table 5).

In selecting the vapor-heat temperatures and exposure periods for any particular seed problem it is essential to employ those that retard germination the least and yet are efficacious. If germination is retarded by the treatment deemed necessary to effect disinfection, the seeds should subsequently be treated with a suitable protectant to avoid damping-off during the lengthened pre-emergence period.

The effect of age of seed on susceptibility to injury by vapor-heat.—It may be presumed that any circumstance that naturally weakens the viability of seeds would lend to proportionately greater injury from vapor-heat treatment. Aging of seed is a common circumstance that militates against good germination. One test with cauliflower seed representing 0, 1, 2, and 3 years of aging respectively, reported in table 5, shows that injury to cauli-

TABLE 4.—*The effect of vapor heat on the germination of certain seeds*

Seed and series number[a]	Vapor-heat treatment		Germination in laboratory[b]	Germination in greenhouse	
	Approach period	Standard period		Seedlings emerged[c]	Mean emergence period
			Per cent	*Per cent*	*Days*
Table beet					
1, a	....	Not treated	77	94	....
1, b	....	145° F., 15 min.	77	.. ....	....
1, c	....	145° F., 30 min.	79	... .	....
1, d	....	145° F., 45 min.	74	72	....
1, e	....	145° F., 60 min.	77	53	....
1, f	....	145° F., 90 min.	68	52	... .
1, g	....	150° F., 20 min.	89	138	....
1, h	....	150° F., 30 min.	78	.. ....	....
1, i	....	150° F., 45 min.	70	....	....
1, j	....	150° F., 60 min.	83	80	... .
1, k	....	150° F., 90 min.	72	. ....	....
1, l	....	155° F., 15 min.	87	..	. ...
1, m	....	155° F., 30 min.	72	... .	....
1, n	....	155° F., 45 min.	72	.. ....	....
1, o	....	155° F., 60 min.	78	.. . ..	.. ..
1, p	....	160° F., 20 min.	76	. .	... .. .
1, q	....	160° F., 40 min.	46	2	. ... ..
1, r	....	160° F., 60 min.	9	0	. ... ..
1, s	....	160° F., 80 min.	1	0	... ...
1, t	....	165° F., 20 min.	70	.. ....	.... . .
1, u	....	165° F., 40 min.	38	... ...	. ... ..
1, v	....	165° F., 60 min.	11	.. ....	. ... . .
2, a*	....	Not treated	.	91	..
2, b*.	130° F., 15 min.	140° F., 30 min.	.. ...	89	....
3, a	....	Not treated	.. ..	168	5.5
3, b	130° F., 15 min.	140° F., 45 min.	..	97	6.5
4, a	....	Not treated	. .	130	8
4, b	135° F., 15 min.	140° F., 60 min.	.. ..	134	8
5, a	....	Not treated	. ....	162	5.5
5, b	130° F., 10 min.	145° F., 30 min.	.... ..	157	5.5
6, a	....	Not treated	.....	79	. . ...
6, b	135° F., 15 min.	145° F., 45 min.	....	85	... .
7, a	....	Not treated	....	256	4
7, b	135° F., 15 min.	146° F., 30 min.	.... .	196	5.5
8, a	....	Not treated	. .	180	5
8, b	135° F., 20 min.	146° F., 45 min.	. ...	147	6.5
Sugar beet					
9, a	....	Not treated	89	179	....
9, b	130° F., 15 min.	140° F., 30 min.	90	185	.. ..
10, a	....	Not treated	....	226	5.5
10, b	130° F., 15 min.	140° F., 35 min.	....	237	5
Carrot					
11, a	....	Not treated	....	45	6.5
11, b	135° F., 15 min.	146° F., 30 min.	....	33	7.5
12, a	....	Not treated	....	46	7.5
12, b	135° F., 20 min.	146° F., 45 min.	.... .	17	9
13, a	....	Not treated	....	75	13
13, b	....	150° F., 45 min.	....	40	17.5
14, a	....	Not treated	....	45	6.5
14, b	135° F., 20 min.	146° F., 30 min.	....	33	7.5

TABLE 4.—(*Continued*)

Seed and series number[a]	Vapor-heat treatment		Germina-tion in laboratory[b]	Germination in greenhouse	
	Approach period	Standard period		Seedlings emerged[c]	Mean emergence period
			Per cent	*Per cent*	*Days*
15, a	....	Not treated	...	46	7.5
15, b	135° F., 20 min.	146° F., 45 min.		17	9
16, a	....	Not treated		75	13
16, b	....	150° F., 45 min.	. . .	40	17.5
Cabbage					
17, a	. . ..	Not treated	95	84	....
17, b	. . . .	145° F., 30 min.	96.5	... .	.. ..
17, c	... .	145° F., 45 min.	97	.. ..	....
17, d	....	145° F., 60 min.	95	. .....	... .
17, e	....	150° F., 30 min.	93	... .	. .
17, f	....	150° F., 60 min.	88.5	. ....	. . .
17, g	.. ..	150° F., 90 min.	66.5	..	. . .
17, h	135° F., 20 min.	145° F., 45 min.	82.7	88	. .
17, i	130° F., 20 min.	150° F., 30 min.	89	52	. .
17, j	135° F., 20 min.	150° F., 60 min.	70.5	40	.. .
17, k	130° F., 20 min.	150° F., 90 min.	23.5	6	.... .
17, l	130° F., 20 min.	150° F., 120 min.	5	8	. .
Brussels sprouts					
18, a	... ..	Not treated	......	76	6.0
18, b	132° F., 15 min.	137° F., 20 min.	. ....	79	6.0
19, a	. . .	Not treated	.. .	89	6.5
19, b	137° F., 15 min.	147° F., 30 min.	..	28	11
Cauliflower					
20, a	... ...	Not treated	93	....	... .
20, b	.. ..	135° F., 30 min.	91	... .	... .
20, c	-	140° F., 30 min.	91	....	... .
20, d	.. . . ..	148° F., 30 min.	32.4	. ....	. ...
21, a	....	Not treated	71	. ....	. . .
21, b	135° F., 20 min.	145° F., 45 min.	0	.. ....	. ...
Peas					
22, a	... ...	Not treated	94.5	....	... .
22, b	... .	160° F., 20 min.	90	....	....
22, c	.. ..	160° F., 40 min.	76.5	....	. ...
22, d	. . . .	160° F., 60 min.	66	....	... .
22, e	. . ..	160° F., 80 min.	55.5	. .....	.
Beans					
23, a	.. ..	Not treated	......	95	6
23, b	. ...	146° F., 60 min.	......	92	6.5
24, a	....	Not treated	......	97	9.5
24, b	....	150° F., 45 min.	......	66	11

[a] An asterisk indicates that the seeds in this series were dipped in a spore suspension of *Phoma betae* before treatment.

[b] Average germination, in per cent, of 2 samples of 100 seeds, or seed-balls, each as determined by standard laboratory seed analysis methods.

[c] Seedlings emerged in percentage of seeds, or seed-balls, sown.

flower seed increases with age and that the process is more suitable for current-season seed. The germination of the fresh seeds treated at the higher temperature level was from 3 to 28 times that of the 3-year-old seed. The strain Snowball "A" was consistently more resistant to injury than Snowball "X."

The proper interval between harvesting seeds and vapor-heat treatment has not been investigated. This relationship should be determined for various kinds of seeds and the diseases associated with them.

The relation of depth of seeds to treating efficiency.—We had hoped that vapor-heat would penetrate sufficiently to permit treating of seeds in sacks. That treatment in sacks is impractical was proved by determining how far

TABLE 5.—*The effect of vapor-heat on the germination of cauliflower seed of different ages, 1946*

Year seed grown	Vapor-heat treatment	Average germination of two strains of seed as determined by standard laboratory methods	
		Snowball "A"	Snowball "X"
		Per cent	*Per cent*
1942	Not treated	90	97
Do	135° F., 30 min.	91	95
Do	140° F., 30 min.	91	95
Do	148° F., 30 min.	20	2
1943	Not treated	93	88
Do	135° F., 30 min.	87	90
Do	140° F., 30 min.	92	91
Do	148° F., 30 min.	54	35
1944	Not treated	95	91
Do	135° F., 30 min.	91	95
Do	140° F., 30 min.	94	92
Do	148° F., 30 min.	46	0
1945	Not treated	94	99
Do	135° F., 30 min.	97	94
Do	140° F., 30 min.	92	95
Do	148° F., 30 min.	73	56

within a mass of beet fruits complete disinfestation was obtained. The beet seed-balls used, as reported in table 6, were contaminated randomly with species of *Stemphyllium, Macrosporium,* and *Penicillium.* Decontamination is significantly reduced at distances of more than one inch within the seed mass even in the case of a favorable subject such as beet seed-balls. The self-insulation against heat provided by small seeds in masses would obviously be more pronounced. This relatively poor penetration must be considered in planning commercial applications of vapor-heat and is discussed later in this paper.

The absorption of water by seeds during vapor-heat treatment.—Seeds treated with hot water absorb from ten to twenty times as much water as those treated by vapor-heat (Table 8). This is a significant advantage of vapor-heat over the hot water method. While all vapor-heat-treated seeds

TABLE 6.—*The relation of the depth of table beet seed-balls in containers to decontamination by vapor-heat*

Depth of seed in trays in millimeters	Vapor-heat treatment		Number of seed-balls cultured	Percentage of seed-balls harboring viable fungi after treatment
	Approach period	Standard period		
		Not treated	352	97
2 to 5	135° F., 15 min.	140° F., 30 min.	30	0
25	130° F., 15 min.	140° F., 30 min.	34	44
75	130° F., 10 min.	140° F., 30 min.	15	93
2 to 5	135° F., 15 min.	145° F., 30 min.	30	0
25	135° F., 15 min.	145° F., 30 min.	42	28
75	135° F., 15 min.	146° F., 30 min.	30	70
2 to 5	135° F., 15 min.	145° F., 45 min.	10	0
25	135° F., 20 min.	145° F., 45 min.	50	4
50	135° F., 20 min.	146° F., 45 min.	15	40
95	135° F., 20 min.	146° F., 45 min.	14	64
2 to 5	135° F., 20 min.	150° F., 30 min.	8	0
25	135° F., 20 min.	150° F., 30 min.	20	0
2 to 5	135° F., 20 min.	150° F., 60 min.	8	0
25	135° F., 20 min.	150° F., 60 min.	30	0
2 to 3	135° F., 20 min.	150° F., 90 min.	8	0
25	135° F., 20 min.	150° F., 90 min.	10	0
2 to 3		145° F., 15 min.	8	50
25		145° F., 15 min.	10	30
2 to 3		145° F., 30 min.	9	0
25		145° F., 30 min.	20	20
2 to 3		150° F., 120 min.	8	0
20		150° F., 120 min.	20	10

appear only slightly dampened, those with porous coats, such as beet seed-balls, absorb more moisture during treatment than seeds with hard "flinty" coats, such as cabbage.   Most treated seeds were almost dry enough to "pour" when removed from the machine.   The moisture content can be

TABLE 7.—*The control of Phoma betae in naturally infected sugar beet seed-balls by vapor-heat treatment*[a]

Series	Treatment	Emergence per 100 seed-balls	Seedlings "damped-off" after emergence	Seedlings with root lesions[b]	Total seedlings infected
			Per cent	Per cent	Per cent
1, a	None	179	50.6	30.1	80.7
1, b	Vapor-heated (130° F. for 10 min. + 145° F. for 30 min.)	185	2.7	3.9	6.6
2, a	None	142	53.5	28.9	82.4
2, b	Vapor-heated (130° F. for 10 min. + 145° F. for 30 min.)	189	1.8	0.6	2.4

[a] The seeds were vapor-heat treated by us and forwarded to Dr. L. D. Leach who carried on the emergence studies.

[b] All seedlings with root and hypocotyl infections were cultured by Dr. Leach to verify *Phoma betae* as the causal organism.

TABLE 8.—*The percentage of moisture absorbed by certain seeds during vapor-heat treatment as compared with that absorbed during hot-water treatment*

Seed	Treatment	Exposure temperature in degrees Fahrenheit and time in minutes	Average[a] percentage of moisture absorbed during treatment expressed as percentage of original dry weight of seed
Table beet	Hot-water	134° F. for 10 minutes	98.8
Do	Vapor-heat	130° F. for 10 minutes + 145° F. for 30 minutes	4.1
Do	Vapor-heat	135° F. for 15 minutes + 140° F. for 60 minutes	5.9
Cabbage	Hot-water	122° F. for 15 minutes	27.0
Do	Vapor-heat	130° F. for 10 minutes + 145° F. for 30 minutes	2.8

[a] Average of 4 replicates.

lowered to the initial dryness by introducing hot air into the chamber before removing the seeds. Proper mechanical equipment for vapor-heat treatment of seeds would dry the seeds automatically before they are stored.

DISCUSSION

The foregoing data indicate that the vapor-heat principle may be used to disinfect and disinfest seeds without serious injury. In many cases reported above, effective treatment was accomplished at levels far below the point of injury to the seeds. Precise temperature control is not a critical factor and the treatment periods are not excessively long. It is likely that vapor-heat treatments can be designed that will effectively replace hot-water treatment of seeds in those cases where thermal disinfection has proved necessary.

The data presented show that because of irregular and inadequate penetration seeds could not be treated in sacks. Practical application therefore would require the construction of special vapor-heat machinery not unlike some of the equipment already used by canners. The seeds could be carried along on slowly moving belts through tunnel-like channels wherein the vapor-heat would be applied. In the last section of the tunnel they could be exposed to warm, dry air so that they would emerge dry enough for final storage. The amount of moisture left on the seed could be so adjusted that a suitable post-emergence protectant could be more effectively applied before they are placed in containers for marketing.

SUMMARY

A method for disinfecting seeds by vapor-heat is described.

Tabulated data presented indicate that certain fungi are killed at relatively low vapor-heat temperatures for short exposures. However, most bacteria are not killed by vapor-heat treatments that are safe for seeds, and the control of bacterial seed-borne diseases by vapor-heat alone seems impractical.

Certain kinds of seeds will apparently endure considerably higher vapor-heat temperatures than are required to kill all associated fungi.   Thus, table beet seed-balls will evidently tolerate a vapor-heat temperature of around 145° F. for as long as 30 minutes without significant reduction or retardation of germination.   *Phoma betae*, an associated pathogen, is killed at 135° F. for 20 minutes, which is about 10 degrees F. lower than the phyto-lethal temperature.

The practical control of *Phoma betae* on and within sugar beet and table beet seed-balls is indicated by these investigations.   Preliminary tests also indicate that the method will kill sclerotia of *Sclerotinia* occurring as a contaminant of cabbage seed.

The important advantages of vapor-heat treatment are (a) temperature control is not critical as for hot water treatment, (b) treatment periods are short in comparison with dry heat, and (c) the difficulty of drying seed which limits the use of hot water is eliminated by proper vapor-heat procedures.

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EFFECT OF SEVERAL SEED PROTECTANTS ON EMERGENCE AND STAND OF OKRA[1]

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(Accepted for publication October 14, 1947)

During the summer months in the coastal area of South Carolina, okra is the most dependable vegetable for the home garden. A considerable acreage is also grown for canning near Charleston. A satisfactory stand of okra plants is often difficult to obtain. Since information regarding okra seed treatment was lacking, experiments with seed protectants[3] were conducted. Results indicate that the use of such materials aids greatly in securing satisfactory stands. A brief summary of the results with the most promising materials has been presented in South Carolina Agricultural Experiment Station Bulletin 361.

One experiment in 1942, two in 1943, and one in 1944 were made in sandy loam soil. Each test was made in a different field. A randomized block design with 5 or 6 replicates of each treatment was used for each test. On each plot consisting of a single row 10 or 12 feet in length, 100 seeds were planted. Clemson Spineless okra seed was used exclusively in all experiments except No. II in which several varieties were included.

Experiment I.—Seed was treated on March 10 and kept in paper bags until planted on March 31, 1942. This was about three weeks earlier than the normal seeding date. The plants that emerged were counted on April 23 and the surviving plants on April 29 (Table 1). The germination of the seed used was low, which seems to be normal for many lots of okra seed.

A second test, similar in design and materials to the first one, was seeded on August 11, 1942. Plants were emerging on August 17. Stand data (Table 1), taken on August 26, indicate the value of the various treatments.

Experiment II.—Spergon and New Improved Ceresan, which appeared to be beneficial in 1942, were tested on seed of six varieties of okra in 1943. The seed was treated on March 4 and kept in closed containers for 24 hours. Each plot, a single 12-foot row, was seeded on March 24.

The average number of plants, for each variety and treatment, emerged by April 12 is shown in table 2. Both Spergon and New Improved Ceresan caused highly significant increases in stand from certain seed lots in com-

TABLE 1.—*Relation of treatment of okra seed to stand, 1942*

	Average number of plants per plot[b]		
	Test 1		Test 2
Treatment[a]	Emerged	Survived	Emerged
	April 23	April 29	August 26
None (Control)	8.3	6.3	55.8
New Improved Ceresan, 0.5 per cent	32.7**	19.2**	73.8**
Spergon, 1 per cent	27.2**	24.2**	71.7**
Spergon, 0.5 per cent	.. . .	... . .	69.7**
Arasan, 0.5 per cent	29.8**	22.8**	64.5*
Arasan, 0.2 per cent	24.2**	16.2**	....
2 per cent Ceresan, 0.5 per cent . .	24.3**	16.5**	. .
Tribasic Copper Sulfate, 2 per cent	22.0**	13.5*	.. . .
Cuprocide, 2 per cent	15.5*	13.2*	.
Semesan, 0.5 per cent	20.0**	12.7*	.. ..
Zinc oxide, 2 per cent	16.0*	10.8	. . ..
Spergonex, 2 per cent	8.0	6.0	. . .
Soaked in water for 20 hours ..	... .	. . ..	32.3
Difference required for significance			
at 5 per cent level	6.7	5.8	8.5
at 1 per cent level	8.7	8.2	11.4

[a] Rate of application is expressed as percentage of weight of seed.
[b] Figures marked with one and two asterisks are significantly higher than those for the untreated seed at the 5 per cent and 1 per cent levels, respectively.

parison with the untreated controls. At the dosages used, there were no significant differences between the means for the two materials.

Experiment III.—A dosage series was tried in 1943 in order to learn

TABLE 2.—*Relation of treatment of seed of okra varieties to stand, 1943*

	Average number of plants emerged on April 12[a]		
Variety	Untreated	New Improved Ceresan, 0.5 per cent	Spergon 1 per cent
1. Clemson Spineless	46.6	67.2**	68.4**
2. Carrother's Green Velvet	18.0	13.6	15.6
3. Louisiana White Velvet	62.8	68.6	76.8**
4. Asgrow White Velvet	34.8	41.2	41.6
5. Dwarf Green	49.4	55.0	52.2
6. Perkin's Mammoth	49.2	50.8	51.2
Difference required for significance			
at 5 per cent level	8.8	8.8	8.8
at 1 per cent level	11.6	11.6	11.6
Mean for treatments	43.5	49.4**	51.0**
Difference between treatment means required for significance			
at 5 per cent level			3.58
at 1 per cent level			4.74

[a] Figures marked with two asterisks are significantly higher at the 1 per cent level than those for the untreated seed.

TABLE 3.—*Relation of the treatment of Clemson Spineless okra seed to stand, 1943*

Treatment	Average number of plants emerged on April 29[a]			
	Dosage, percentage by weight			Mean
	0.25	0.5	1	
New Improved Ceresan	82.6	76.6	76.0	78.3**
Spergon	74.4	74.2	75.8	74.8*
Arasan	76.6	71.4	74.0	74.0*
Fermate	75.2	69.6	76.2	73.7*
2 per cent Ceresan	70.4	74.8	72.2	72.5
Semesan	68.4	65.8	66.6	66.9
None (Control)	....	....	....	68.6
Soaked in water for 3 hours	... .	.. ....	....	58.8
Mean for dosage[b]	74.6	72.1	73.5	.. . .

Difference required for significance at 5 per cent level	4.98
at 1 per cent level	6.63

[a] Figures marked with one and two asterisks are significantly higher than those for the untreated seed at the 5 per cent and 1 per cent levels, respectively.
[b] No significant difference between means for dosages.

TABLE 4.—*Relation of treatment of Clemson Spineless okra seed to seedling emergence, 1944*

Treatment		Average number of plants per plot[a]
Material	Dosage (Percentage by weight)	Emerged May 2
None (Control)	... .	' 38.6
Spergon	4	40.8
Do	2	58.6*
Do	1	57.6*
Do	0.25	57.2*
Do	0.062	44.2
Arasan	2	51.8
Do	1	54.2
Do	0.25	56.0*
Do	0.062	52.2
2 per cent Ceresan	1	46.4
Do	0.25	52.6
Do	0.062	53.6
Do	0.016	52.8
New Improved Ceresan	1	49.6
Do	0.25	52.2
Do	0.062	60.0*
Do	0.016	37.4
Fermate	2	46.6
Do	1	52.0
Do	0.25	48.8
Do	0.062	48.8
Phygon	0.25	48.2
Seed Disinfectant No. 5	0.25	45.4
Do No. 6	0.25	37.6

Difference required for significance	
. at 5 per cent level	17.4
at 1 per cent level	23.0

[a] Figures marked with an asterisk are significantly higher at the 5 per cent level than those for the untreated seed.

the relative effectiveness of three rates of application of several materials in increasing the stands. Seed was treated on March 23, kept in closed glass containers for 24 hours, and planted on April 9. Treatment with New Improved Ceresan at 0.25 per cent dosage resulted in the best stand (Table 3). The differences between the means for dosages were not significant.

Experiment IV.—Seed of the Clemson Spineless variety and the required amount of the dry chemical for each treatment listed in table 4 were mixed together in an electric rotary mixer at 30 to 50 r.p.m. for 4 minutes on February 9, 1944. Soil moisture was optimum at planting on April 7 but was unusually high for several weeks that followed. Counts of the plants emerged by May 2 are given in table 4.

SUMMARY

Treatment of okra seed with either New Improved Ceresan or Spergon resulted in a significant increase in stand in each of five tests. In the tests made, treatment with New Improved Ceresan at the rate of 4 ounces, Spergon at 16 ounces, or Arasan at 4 ounces per 100 pounds of seed was beneficial, whereas treatment with Fermate, 2 per cent Ceresan, or Semesan was beneficial to a lesser degree. In preliminary tests tribasic copper sulfate, Cuprocide, zinc oxide, Phygon, Seed Disinfectant No. 5, Seed Disinfectant No. 6 and Spergonex were not very promising as protectants for okra seed. Soaking the seed in water for several hours prior to seeding resulted in a decrease in the stand.

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A COMPARISON OF HOME–MADE BORDEAUX MIXTURE WITH OTHER FUNGICIDES FOR CONTROL OF SCAB ON THE SCHLEY AND MOORE VARIETIES OF PECAN

JOHN R. COLE[1]

During the past 12 years, 1935–1946, the writer has used a number of fungicides (Table 1) in comparison with home-made Bordeaux mixture[2] in order to control scab (*Cladosporium effusum* (Wint.) Demaree), a disease that is one of the limiting factors in pecan production.

The tests were made on the Schley variety of pecan in south Georgia, as well as the Moore variety in north Florida. The Schley is highly susceptible to the scab disease in the locality where the tests were conducted. While the Moore is classed as resistant to scab in most localities, it is susceptible in north Florida.

Each test plot contained a minimum of 9 trees, with an average of 12. A high-power spray machine that maintained a pressure of 400 to 600 pounds with the spray gun open was used in making the 4 spray applications on all test plots.

The purpose of these tests was to find a fungicide that would give commercial control of scab or compare favorably with home-made Bordeaux mixture, an effective and economical fungicide but one that apparently has a tendency to increase the infestation of the black pecan aphid, *Melanocallis caryaefoliae* (Davis).

Table 1 indicates that of the various fungicides used in spraying the Schley variety to control scab during the period 1935–1946, inclusive, Bordeaux mixture gave a greater increase in yield over the unsprayed checks than other fungicides that were tested. In addition, with the exception of 1943 when the check trees were accidentally sprayed one time, the trees sprayed with Bordeaux mixture always gave an increase in yield over the unsprayed checks. Likewise, in 1943 when the check trees gave a higher yield of nuts than the ones that were sprayed 4 times, the trees sprayed with Bordeaux mixture produced more nuts than those trees sprayed with Fermate, or Fermate plus lime.

The set of nuts was greater on the check trees in 1943 than it was on the sprayed ones. Under favorable weather conditions, and with good nutrition of the check trees, *i.e.*, very little premature defoliation from the scab disease and no second growth the preceding year, the check trees occasionally bloom more heavily than the sprayed ones, especially in the "off-

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TABLE 1.—*Fungicides used in past 12 years for control of pecan scab on the Schley and Moore varieties at Albany and Fort Valley, Georgia, and Monticello, Florida*

Varieties and treatment	Amount of fungicide in 100 gal. of water	Year treated	Av. yield of nuts per tree	Increase or decrease in yield of sprayed trees over unsprayed checks
			lb.	lb.
Schley Variety				
Bordeaux mixture	6–2	1935	30	15
	(6 copper-2 lime)			
Zeolite (sodium silicate)	3 lb.	do	17	2
Coposil (copper silicates and zinc)	5 lb.	do	11	– 4
Copper phosphate (Cu₃(PO₄)₂·3H₂O)	4 lb.	do	15	. . .
Check	. ...	do	15	.
Bordeaux mixture	6–2	1936	55	41
Tribasic copper sulfate (53 per cent metallic copper- basic copper sulfate) .. .	6 lb.	do	19	5
Wettable sulfur (S)	6 lb.	do ·	15	1
Check	.	do	14	. .
Bordeaux mixture	6–2	1937	73	66
Cupro-K (copper oxy- chlorides)	6 lb.	do	14	7
Check	. ... ..	do	7	.
Bordeaux mixture	6–2	1938	37	22
Tenn. Copper 53 per cent (basic copper sulfate)	3 lb.	do	20	5
Tenn. Copper 26 per cent (basic copper sulfate) ...	3 lb.	·do	18	3
Copper oxalate (CuC₂O₄·½H₂O)	4 lb.	do	14	– 1
Check	. ...	do	15	.
Bordeaux mixture	6–2	1939	62	58
Cuprocide (cuprous oxide) ...	3 lb.	do	8	4
Check	....	do	4	. .
Bordeaux mixture	6–2	1940	34	7
Yellow copper oxide (yellow cuprous oxide)	1½ lb.	do	25	– 2
Cuprocide 54Y (yellow cuprous oxide)	3 lb. + 1 qt. oil	do	30	3
Check	....	do	27	. ...
Bordeaux mixture	6–2	1941	81	58
Sodium polyphosphate (poly- phosphates of sodium)	4 + 1 lb. lime	do	36	13
Copper oxychloride sulfate (copper oxychlorides and sulfates)	4 + 1 lb. lime	do	27	4
COCS 65 (copper oxychloride- copper basic sulfates)	6 lb.	do	39	16
Copper Hydro (copper basic salts)	5 lb.	do	25	2
Check	... .	do	23	.. ....
Bordeaux mixture	6–2	1942	62	41
Fermate (ferric dimethyldi- thiocarbamate)	3 lb.	do	44	23
Check	....	do	21	. ....
Bordeaux mixture	6–2	1943	51	......
Fermate	3 lb.	do	37	......
Fermate + Lime	3 lb. + 2 lb. lime	do	35	......
Check .... ·...	....	do	73ᵃ	.. ....

TABLE 1.—(Continued)

Varieties and treatment	Amount of fungicide in 100 gal. of water	Year treated	Av. yield of nuts per tree	Increase or decrease in yield of sprayed trees over unsprayed checks
			lb.	lb.
Bordeaux mixture	6–2	1944	40	29
Check		do	11	
Bordeaux mixture	6–2	1945	20	19
Dithane (disodium ethylene bisdithiocarbamate (dry))	3 lb.	do	5	4
Dithane, Zinc sulfate, and Lime	3-1-½ lb.	do	6	5
U. S. Rubber No. 604—Phygon (2,3 dichloro naphthoquinone 1,4)	3 lb.	do	3	2
Puratized N5-E (phenyl mercuri triethanol ammonium lactate)	1: 15,000	do	5	4
Puratized N5-E	1: 20,000	do	4	3
Isothane Q-15 (lauryl isoquinolinium bromide)	1 pt.	do	2	1
Check		do	1	.
Bordeaux mixture	6–2	1946	27	27
Bordeaux mixture + Omilite Polyethylene polysulfide (Omilite)	1 qt.	do	17	17
Puratized N5-E	2½ qt.	do	1	1
337 (1-hydroxyethyl-2-heptadecyl glyoxalidine)	1 qt.	do	4	4
Do	1 gal.	do	2	2
341 (2-heptadecyl glyoxalidine)	2 gal.	do	3	3
Do	1 gal.	do	1	1
Check	4 gal.	do	2	2
		do	0	
Moore Variety				
Bordeaux mixture	6–2	1946	77	22
Bordeaux mixture + Dupont spreader-sticker	4 oz.	do	87	32
Copper A (2–5 Cu(OH)₂·CuCl₂(CuO)ₓ)	3 lb.	do	94	39
Copper A + Dupont spreader-sticker	4 oz.	do	92	37
Fermate	3 lb.	do	83	28
Fermate + Dupont spreader-sticker	4 oz.	do	83	28
Check		do	55	..

ᵃ Check trees accidentally sprayed one time—third application—with Bordeaux mixture.

year'' of the sprayed trees after these have had the stress of a heavy crop the preceding season. Furthermore, the daily distribution of rainfall was less than normal in 1943. This condition retarded the progress of the scab disease.

On the Moore variety in 1946 both Copper A and Fermate, with and without Dupont spreader-sticker, gave a greater increase in yield over the unsprayed check trees than did the Bordeaux mixture without the spreader-

sticker. This was probably because of the location of the trees, since the topography of the orchard prevented the randomization of the plots. However, the scab infection during the growing season, as well as quality of the nuts at harvest, indicated that there was no significant difference in the quality of the nuts from plots sprayed with Copper A, Fermate, or Bordeaux mixture, either with or without spreader-stickers.

In contrast to results with Copper A and Fermate, the addition of the spreader-sticker to the Bordeaux mixture increased the yield of nuts. The quality of the nuts was not affected, however.

The daily distribution of rainfall was above normal for 1946 at Albany, Georgia, but it was below normal at Monticello, Florida, which accounts for the high yield of nuts from the check trees at Monticello.

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NATURAL INFECTION OF REPLANTED APPLE TREES BY WHITE ROOT ROT FUNGUS

J . S . C o o l e y [1]

In the course of studies on root diseases of apple trees during the past 15 years the white root-rot fungus, *Corticium galactinum* (Fries) Burt, while relatively infrequent in occurrence, has been noted[2] to cause the death of many trees in the affected orchards. This paper records the results of replanting apple trees where trees affected with white root rot have been removed. It also discusses the source, persistence, and distribution of the infective material and the conditions favoring the infection of apple trees.

REPLANTING EXPERIMENTS

Replanting experiments have been carried on in 2 orchards where heavy losses from the disease have occurred. One of these orchards, located at Middletown, Virginia, in the Shenandoah Valley, contained 252 trees of the York Imperial, Grimes Golden, Winesap, and Stayman Winesap varieties that were 27 years old at the beginning of the experiment in the spring of 1937. This orchard was on land cleared of hardwood trees, predominantly white oak (*Quercus alba* L.).

The other experimental orchard, located at Heards, Virginia, in the Piedmont, contained 80 trees of the Winesap and Yellow Newtown varieties. This plot of 80 trees was part of a 50-acre orchard which was 18 years old in the spring of 1937 and was on land from which deciduous trees, predominantly chestnut and some oak, had been cleared.

At the beginning of the experiment there were gaps in the original stand of trees in both orchards. A detailed survey was made in 1937 to ascertain the number and location of the original trees killed by the white root-rot fungus. Two-year-old Delicious trees on seedling rootstocks were used for replanting both orchards in 1937, 1939, and 1940. Since the location of each original white root-rot case was known, the replanting experiments, it was believed, would yield pertinent information on the fate of young trees planted in foci of white root-rot infection.

In the replanting operations 3 methods were followed: (1) either 2 or 3 bushels of rotted manure were incorporated in soil in which the young tree was planted; (2) a 4-inch layer of coarse manure was placed at the bottom of the 20-inch-deep hole, a layer of soil was added, and the tree was planted and mulched with coarse manure; (3) the tree was replanted in soil without the addition of any manure.

[2] Cooley, J. S., and Ross Davidson. A white root rot of apple trees caused by *Corticium galactinum*. Phytopath. 30: 139–148. 1940.

The results of the replanting tests are in table 1. Because of droughts and the lack of cultivation the replants had unfavorable growing conditions, and the mortality was greater than should be expected. The data recorded in the table show the condition of the original apple trees and of the replants in the Heards orchard in the summer of 1943 and in the Middletown orchard in 1944.

Tree growth was best where replanting method No. 2 was followed and poorest with method No. 1. In spite of variations in growth response, no relationship was found between replanting method and subsequent infection by the white root-rot fungus.

TABLE 1.—*Loss of replanted apple trees where white root rot is present in the orchard*

Original trees						Replants (set in 1937)		
Condition at replanting time in 1937			Condition in 1944			Condition in 1944		
Trees alive (per cent)	Trees dead from white root rot (per cent)	Trees dead from other causes (per cent)	Trees alive (per cent)[a]	Trees dead from white root rot subsequent to 1937 (per cent)[a]	Trees dead from other causes subsequent to 1937 (per cent)[a]	Trees alive (per cent)[b]	Trees dead from white root rot (per cent)[b]	Trees dead from other causes (per cent)[b]
Orchard at Middletown, Va.								
66.6	14.3	19.1	58.3	3.5	4.8	66.7	17.8	15.5
Orchard at Heards, Va.[c]								
58.7	15.0	26.3	48.7	5.0	5.0	54.5	27.3	18.2

[a] Based on original stand of 252 trees in the Middletown orchard and of 80 trees in the Heards orchard.

[b] Based on 84 replants in the Middletown orchard and 33 in the Heards orchard. Of the 84 replants in the Middletown orchard 36 were in white root-rot-infested sites, and of these, 15 died of white root rot by August, 1944. Of the 33 replants in the Heards orchard, 12 were in white root-rot-infested sites, and of these, 9 died of white root rot by July, 1943.

[c] Final record taken in 1943.

Further observations were made on the Middletown orchard in August, 1946, when more of the original trees had died of the disease. By this time some of the replants set where the original trees were affected with white root rot were beginning to bear. In other respects there was little change since 1944. The data indicate that apple trees set where white root rot was present are likely to succumb to the disease. As many as one-half such replants were affected by that disease in the period of 7 or 8 years. However, some of the trees that were set where the originial tree was known to have been removed because of white root rot have grown normally and seem healthy after 7 to 10 years. The best replants are where coarse stable manure was put in the bottom of the hole and covered with several inches of soil before the tree was planted.

SOURCE, PERSISTENCE, AND DISTRIBUTION OF INFECTIVE MATERIAL

Closely related to the replanting experiments and furnishing an understanding of some of the results is the study of the source, persistence, and distribution of infectious white root-rot material.

The observations made prior to and during the course of the replanting experiments (a period covering 15 years) help to explain some of these factors. At Middletown, Virginia, for example, the original planting grew satisfactorily until the trees were 15 to 20 years old, and then trees began to die on a clay ridge where the soil was poorer than in the rest of the orchard. The white root-rot fungus was not recognized as being pathogenic to the apple trees at the time, and so it can only be inferred that *Corticium galactinum* was responsible for the killing of the trees. In recent years the large and thrifty but now older trees growing in the better soil are dying of the disease.

Trees scattered over the orchard have continued to die of this disease during the time they have been under close observation. The disease usually attacks at the collar. The fungus, as shown by infected trees, does not radiate out from a focus of infection, as would be the case if the fungus spread through the soil, and this suggests dissemination by wind or manual means. When an apple tree is attacked by this pathogen it usually dies within 2 years after symptoms of the disease appear in its top, which discounts the probability that an affected tree is attacked many years before it dies. The trees in nearby apple orchards planted on land that was not newly cleared have not been affected by this disease. Nevertheless, the pathogen sporulates so freely that there is probably adequate wind dissemination of spores to give infection to nearby orchards, provided the proper substrate is present on which the fungus can become established. Inoculation experiments and observation of diseased trees indicate that for infection to take place in nature, it is necessary to have a reservoir of food on which the fungus can grow, such as woody material left on newly cleared land. Forest-tree stumps, and particularly the deeper roots, may serve for years as a substrate for the fungus; sporulation on diseased trees and their stumps probably provides inoculum for later infection of trees.

These observations indicate that the infection of apple trees by the white root-rot fungus is dependent upon woody material in the soil. This becomes inoculated with the fungus, and from this reservoir of infected material living apple-tree roots become infected. Where this woody debris is lacking, as in an orchard on old land, the fungus presumably fails to become established.

Searches have been made repeatedly for some wild host plant that is particularly susceptible to this disease. No evidence has been found that either the forest trees or the shrubby growth in the forest or other plants growing near an apple orchard are especially susceptible. On two occasions white-oak trees growing in proximity to an apple orchard were affected by

the disease, but in both cases the oaks were suppressed and also were growing in a poorly drained site.

Observations indicate that after a forest tree has been cut down the stump is susceptible to the attack of this fungus, which can live there as a saprophyte until the stump is rotted.

Leach[3] has noted that *Armillaria mellea* does not readily attack a vigorous living tree, but may readily attack a stump. A case has been observed by the present writer where ornamental shrubs were killed by *Corticium galactinum,* which apparently spread from an oak stump to the ornamental plants. At the same time that the shrubs were dying of the disease a black oak (*Quercus velutina*) was growing nearby in the infested soil and showed no evidence of being affected by the disease. The evidence at hand indicates that forest trees and shrubs are very rarely attacked by this fungus. It can, however, live as a saprophyte on stumps of various woody species and shrubby plants when growing in a soil containing inoculum.

SUMMARY

Replanting experiments have been carried on in 2 apple orchards in Virginia in which *Corticium galactinum* white root rot prevailed. Several times during the 8 years of the experiment the dead trees have been removed, the cause of the death of the removed trees determined where possible, other trees planted, and a record kept of the condition of the replant. As many as 15 per cent of the original apple trees of each experimental plot became affected with white root rot. The original trees, which ranged up to 37 years old, continued to die of the disease during the time the plots were under observation. During the period of the experiment, over one-half of the replants in infested soils became infected with white root rot.

Observations on the restricted distribution and the conditions favoring infection and spread of the disease are reported. The general deduction is made that the presence in the soil of woody material is necessary for the start and maintenance of the pathogen as well as for subsequent infection of apple trees.

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CYCLAMEN PETAL SPOT, CAUSED BY BOTRYTIS CINEREA, AND ITS CONTROL

C. M. TOMPKINS AND H. N. HANSEN

INTRODUCTION

A petal-spot disease, affecting commercial varieties of potted Persian cylamen (*Cyclamen persicum* Mill.), has been observed each autumn and winter since 1935 in certain greenhouses in the San Francisco Bay region of California. The conspicuous symptoms decreased the market value of affected plants.

The results of inoculation tests and the method of controlling the disease are briefly discussed in this paper.

REVIEW OF LITERATURE

Although the gray mold disease of cyclamen plants, caused by *Botrytis cinerea* Pers. under conditions of high temperature and high humidity, is well known and frequently destructive on flowers, leaves, and petioles, it was not until 1938 that Wenzl,[1] in Austria, called attention to a hitherto undescribed petal-spot disease, also caused by *B. cinerea,* but prevalent in the cold, wet weather in late summer and fall of 1937.

SYMPTOMS OF THE DISEASE

Wenzl has adequately described and illustrated the symptoms of the disease affecting cyclamen petals, and hence only a brief, supplementary discussion is presented herewith.

The petal spots, at first mere flecks, soon enlarge to form round but more generally elliptical-shaped areas, 1 to 4 millimeters in diameter (Fig. 1 and 2). On pink, red, and salmon-colored varieties of cyclamen, the spots consist of an outer band of living tissue whose color is deeper than the normal color of the petal and a very small center which at first is of normal color, soon becomes water-soaked, and finally tan and necrotic (Fig. 1 and 2). On white-flowering varieties, the outer band of the spot first appears water-soaked while the center is necrotic; later the entire spot becomes necrotic. The spots, which may vary in number from a few to many, are scattered irregularly over the surface of the petal; usually between and with their long axes parallel to the veins. When heavily spotted, petals may be distorted.

All colored and white varieties of cyclamen grown as potted plants in greenhouses for the florists' trade appear to be susceptible when exposed to relatively low air temperatures (45° to 60° F.) and high humidities during the flowering season.

1 Wenzl, Hans. *Botrytis cinerea* als Erreger einer Fleckenkrankheit der Cyclamen Blüten. Phytopath. Ztschr. 11: 107–108. 1938.

FIG. 1.   Flowers of a pink-flowering variety of Persian Cyclamen showing typical spots induced by *Botrytis cinerea* under natural conditions.

Leaf infection may occasionally be observed in conjunction with petal spot, but it is generally confined to older leaves and is of minor importance.
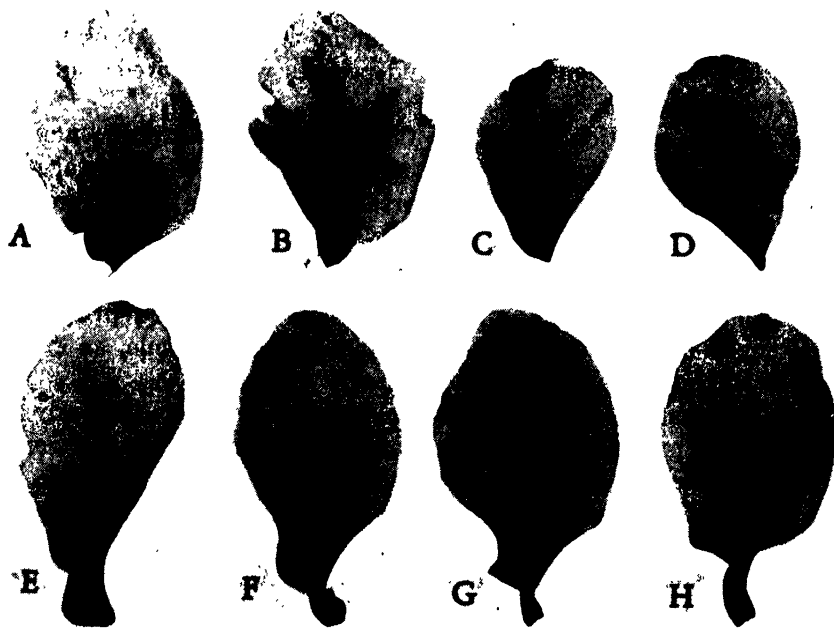

FIG. 2.   Petals of a pink-flowering variety of Persian Cyclamen: A, B, E, F, G, H. Petals showing typical spots induced by *Botrytis cinerea* under natural conditions.   C, D. Healthy petals.

THE CAUSAL FUNGUS, BOTRYTIS CINEREA

Each autumn and winter since 1935, unsuccessful attempts were made to isolate an organism from spotted petals by means of tissue plantings on poured agar in Petri dishes and by placing infected petals in moist chambers. Finally, in 1945, pure cultures of *Botrytis cinerea* were obtained with ease on poured plates and in moist chambers. No valid reason can be given for failure to isolate this fungus in earlier trials.

Healthy white- and colored-flowering varieties of cyclamen were inoculated in a cool greenhouse during the fall and winter months of 1945 and 1946. The potted plants, on saucers, were given a thorough watering late in the afternoon to provide the necessary humidity. Then the upper and lower surfaces of partly and fully expanded petals were atomized with spores of the fungus suspended in sterile-distilled water. Controls were atomized with sterile-distilled water, after which all plants were covered with glass jars. In 1945, a total of 10 white and 16 colored potted cyclamen plants, each with 6 to 10 or more flowers, were inoculated, while a corresponding number of plants served as controls. All petals sprayed with the organism became infected in less than 15 hours, while the controls remained healthy. The symptoms on artificially-infected petals were identical with those of naturally-infected specimens. In 1946, 6 white- and 12 colored-flowering varieties were inoculated; controls consisted of the same number of plants. The results were comparable to those obtained in the preceding tests. The fungus was reisolated from each set of inoculated plants, and the reisolates were identical with the parent culture and proved highly pathogenic. No infection of leaves and petioles was observed while these tests were in progress.

The short incubation period for the disease under experimental conditions is in close agreement with observations made in commercial greenhouses where rapid development and spread of the disease may occur within a matter of hours.

CONTROL OF THE DISEASE

In 1938, before Wenzl's[2] publication was available and prior to the establishment of the true nature of the disease in California, an effective and practical method of control was devised.

It is customary, locally, to grow cyclamen plants in continuously cool, well-ventilated greenhouses. When plants commence to flower in September and October, the humidity is relatively low, and they are free from petal spot. With the advent of the rainy season in November, air temperatures decrease and the relative humidity increases. During the evening hours, apparently the high humidity provides ideal conditions for rapid infection.

The disease may be controlled by changing the environmental conditions surrounding the plants during the evening hours. It was found that by

2 See footnote 1.

closing the top or peak ventilators of the greenhouse at dusk, leaving the side ventilators wide open, and turning on the steam in each of two pipes on opposite sides and traversing the length of the greenhouse, the disease can be entirely prevented. While the heat thus provided is sufficient to reduce the relative humidity to a point unfavorable for infection, it does not materially increase the air temperature. Early the next morning, the top ventilators are opened wide and the steam is turned off. This method then becomes a standard daily practice. Apparently air temperature is of secondary importance, because the disease which has been observed over a wide range of low temperatures may easily be controlled by providing a drier atmosphere.

This method of control has no effect on previously-infected petals. They should be excised from the plants before adopting the method described above in order to observe the full effects on the new flower buds as they open. New petals are always free from spotting.

SUMMARY

Petal spot of Persian cyclamen is prevalent in the late fall and winter months, coinciding with the rainy season, in greenhouses in the San Francisco Bay region of California.

Symptoms of the disease consist of small spots, round to oval, few to many, scattered at random between the veins of the petal. With colored varieties, the spots contain an outer area of deeply-colored tissue with a small, tan, necrotic center. On white varieties, the outer area is at first water-soaked, with a tan, necrotic center; later the entire spot becomes necrotic. No leaf infection occurs.

The disease, first recorded by Wenzl in Austria, is caused by *Botrytis cinerea*.

Healthy cyclamen plants were artificially infected in the greenhouse by atomizing with a spore suspension of the fungus. The incubation period on the petals was less than 15 hours.

The disease is favored by relatively high humidity, accompanied by low air temperatures (45° to 60° F.).

Prevention of the disease depends upon daily closing of the top ventilators of the greenhouse at dusk, leaving the side ventilators wide open, and turning on two steam pipes to provide drier air conditions during the evening hours.

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A PHYCOMYCETE PARASITIC ON APHIDS

M . R . H a r r i s [1]

In the potato growing areas of the State of Maine, aphids are the principal means of spreading virus diseases. During detailed studies on the problem of control of the various virus diseases attention was given to the abundance of aphids. It was observed that frequently fungi were apparently the cause of considerable mortality among the aphids, and an effort was made to isolate the species of fungi present on dead aphids. A collection of fungus-infested aphids was made near Mapleton, Maine.[2] The diseased insects were found on the underside of the older potato leaves. There were four species present: the green peach aphid, *Myzus persicae* (Sulz.); the potato aphid, *Macrosiphum solanifolii* (Ashm.); the foxglove aphid, *Myzus convolvuli* (Kltb.) (*pseudosolani* Theob.); and the buckthorn aphid, *Aphis abbreviata* (Patch). The dead insects were cultured and the fungi isolated were held for study. After the field season, observations on aphids parasitized by fungi were continued in the greenhouse. Isolations of the fungi found on aphids growing on greenhouse plants furnished additional cultures for study. It was during the course of this work that the fungus described in this paper was isolated and later identified.

METHODS AND MATERIALS

In isolating the fungus dead aphids were placed on water agar on the bottom of a sterile Petri dish. Over each insect was placed a sterile Van Tieghem cell. Entomophthorous fungi are known to be phototropic, so the Petri dishes were placed in a cardboard box with light admitted through a small hole in the top. Within a few hours the fungus began to discharge spores against the lower surface of the cover slip on top of the Van Tieghem cell. Within 24 hours the spore mass was dense enough to be seen with the naked eye.

Masses of spores were transferred from the cover slip to four types of media. An effort was made to provide media containing some form of protein. Sawyer (7) working with fungi parasitic on the black-headed fire worms *Ropobota vacciniana* found that among other things the yoke of eggs from which fat had been extracted by ether was an excellent medium for growth. A quantity of such medium was prepared and spores transferred under sterile conditions. Growth was slow with little aerial mycelium but numerous spores were produced. A second medium was made by adding three gm. beef extract and ten gm. gelatin to a liter of water agar. On this

medium the fungus made a slow growth. Later, a third medium was made using ten gm. dextrose in addition to the other ingredients of the second medium. The fungus still made a slow growth. A fourth medium used was malt agar. The fungus grew and sporulated vigorously. Spores were cast in the direction of strongest light and completely covered the under surface of a Petri dish top in a few days.

IDENTIFICATION OF THE FUNGUS

A study of the fungus in culture showed that the mycelium in the medium was much branched but the aerial hyphae were usually unbranched.
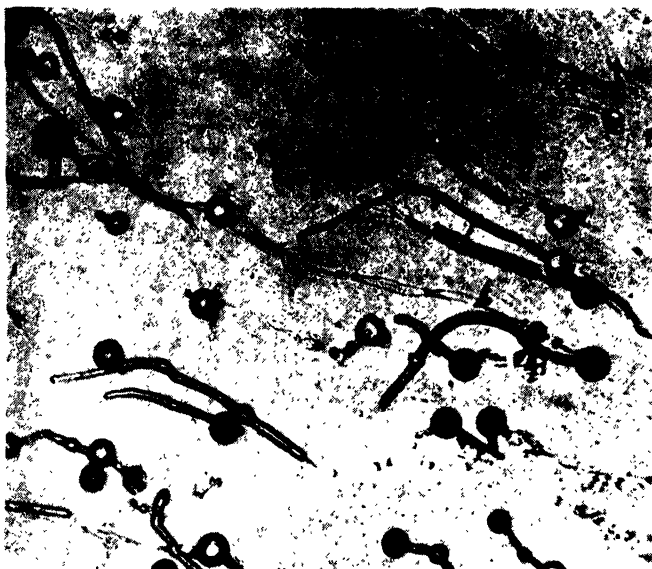

FIG. 1. Germinating conidia of *Entomophthora coronata* showing papillae, secondary conidia, and segments of mycelium both filled with protoplasm and empty. × 100.

The hyphae were rather large and averaged about 12 μ in diameter. The conidiophores were mostly unbranched and erect. They were positively phototropic and similar to the mycelium in size and shape. The conidia were rather large and globose, with a prominent papilla at the base: they averaged about 30 μ in diameter. Under humid conditions and at room temperature, the conidia germinated very easily to give rise to mycelium or secondary conidia (Fig. 1). In old cultures, a number of conidia which behaved as resting spores were observed. These conidia had slightly thicker walls and were somewhat darker than young conidia but their most outstanding characteristic was a covering of blunt hairlike appendages over the entire surface (Fig. 2). Resting spores which had germinated produced conidia similar in appearance to those produced by conidiophores above the mycelial layer. In old cultures the hyphae produced septae making segments which were either empty or filled with protoplasm. Those with proto-

plasm were observed to enlarge somewhat and to take on the appearance of a hyphal body which sometimes resumed growth when conditions were favorable.

This fungus was first observed in the United States by Martin (6) who cultured it out of decayed wood. He applied the name *Conidiobolus villosus*, based on the fact that the genus *Conidiobolus*, founded by Brefeld (1) was distinguished from *Entomophthora* because it is a saprophyte. Later work on the morphology and physiology of the fungus by Kevorkian (5), who found it on living termites in Cuba, showed that it was a parasite as well as a saprophyte. Kevorkian made a critical study of the morphology
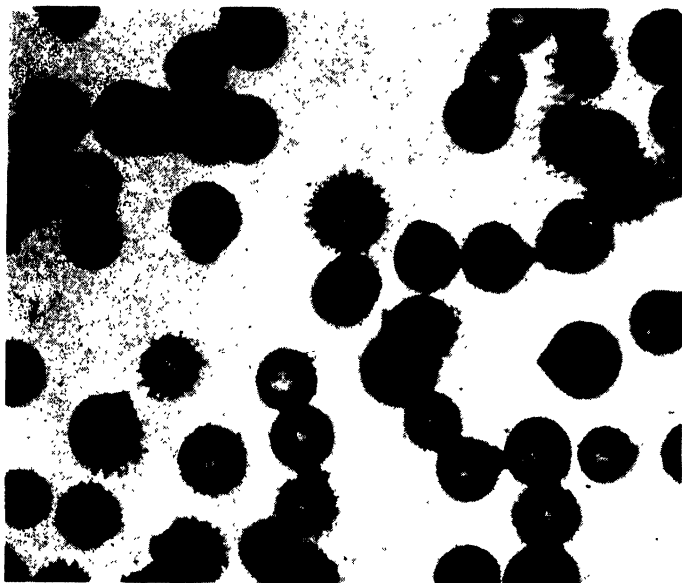

FIG. 2. Conidia of *Entomophthora coronata* in resting stage, covered with blunt hair-like appendages. × 300.

and physiology of the fungus he isolated from termites together with the fungus isolated by Martin. He noted that a similar fungus was described earlier by Gallaud (4), who found spinose resting spores. Constantin (2) identified this fungus as *Delacroixia coronata* (Cost.) Sacc. Kevorkian concluded that the parasitic nature of the organism, not observed by Martin, together with its morphology as described by earlier workers warranted a new combination. He applied the name *Entomophthora coronata* (Cost.) Kevorkian. A study of the fungus found by the writer on the green peach aphid in the greenhouse shows that it is identical with that studied by Kevorkian and because of its parasitic habit should be described as *Entomophthora coronata*. This fungus was also found along with *Empusa aphidis* Hoff. in a collection of dead green peach, potato, and foxglove aphids from a potato field near Mapleton.

<center>INOCULATION STUDIES</center>

In order to determine whether the fungus was pathogenic on the green peach aphid a method of natural inoculation was devised. Cultures of the fungus were grown on malt agar in 50-mm. Petri dishes. When the fungus was sporulating freely cultures were placed directly under potato leaves supporting a heavy aphid population. The Petri dishes were so placed that light came to the fungus through the potato leaf. The fungus cast numerous spores against the lower surface of the leaf. Most of the aphids moved to some other portion of the plant, but there were a small number of aphids remaining which were diseased or dead. Upon being cultured the fungus was recovered.

A second method of exposing aphids to the fungus was devised. Potato leaves were cut from a plant and all but the terminal leaflet removed. Each leaflet was infested with a large population of aphids. The stem was placed in water and the leaflet placed over a Petri dish in which was a vigorously sporulating culture of the fungus. After being exposed to the fungus spores for two days, the aphids were transferred to a potato plant previously free of aphids. The following day and for several days thereafter a number of dead aphids were found on the under side of the potato leaves. Upon being placed on maltose agar they yielded cultures of the fungus. Although a large number of aphids were exposed to the fungus a relatively small proportion of them became infected.

A number of aphids that had been exposed to the fungus were studied under magnification sufficient to enlarge the aphid ten times. Spores were observed clinging to various portions of the aphid body. Since the fungus shows a preference for carbohydrates as a growing medium and since the aphids secrete a carbohydrate substance known as honeydew it seems possible that the fungus may grow into the body of the aphid on food material produced by the insect.

<center>DISCUSSION</center>

The biological control of the aphids in potato fields by means of fungi offers an interesting field of investigation. Dustan (3) has shown that biological control of an insect by means of a parasitic fungus is possible through artificial culture and dissemination of the spores. The results of studies reported in this paper indicate that *Entomophthora coronata* can be cultured rather easily, but only a small percentage of aphids exposed to it become infected. Field observations indicate that the fungus usually does not appear until the aphid population on a plant is very high. Under field conditions this fungus together with other species of entomogenous fungi, given favorable environmental conditions, have destroyed aphids on potato vines late in the season after the aphids were very numerous. By introducing this and other species of entomogenous fungi into potato fields before such fungi normally appear, these studies suggest that aphids possibly could

be infected. An epidemic of fungus infection on the aphids might be started at a period early enough to have a possible value in aphid control.

SUMMARY

1. Under greenhouse conditions, a fungus, *Enthomophthora coronata* (Cost.) Kevorkian has been found to be parasitic on the green peach aphid.

2. The fungus was grown on media containing both proteins and sugars. Best growth was observed on maltose agar.

3. Inoculation experiments suggest that the fungus is pathogenic on aphids.

4. A study of living aphids exposed to the fungus indicates that spores present on the body of the insect may germinate on honeydew secreted by the insect.

5. The study suggests that this fungus may have some value as a natural agency of aphid control.

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PHYTOPHTHORA CINNAMOMI ROOT ROT OF AVOCADOS UNDER TROPICAL CONDITIONS[1]

BOWEN S. CRANDALL[2]

INTRODUCTION

In 1944, soon after the start of organized pathological work at the Estacion Experimental Agricola de Tingo Maria and about 8 years after the opening of the region to colonization, dying and declining avocado trees, *Persea americana* Mill., were observed. The first cases were large, mature trees located on the properties of small landowners. These trees showed clear cases of collar rot followed by quick death and from them *Phytophthora cinnamomi* Rands was isolated. Later the same year the disease was found in seedling and mature avocados being grown by the Station and *P. cinnamomi* was again isolated. Apparently the first recognition of the disease in this area, at least in this part of South America, was reported in 1945 (3). The disease is apparently the limiting factor to avocado growing in this region. Because of the controversial nature of the evidence presented by various workers on the relationship between *P. cinnamomi*, wet soil, and the decline or collapse disease, it was necessary to undertake sufficient work to solve the problem locally, in order that recommendations could be made in regard to the avocado program. The results herein reported are based on observations and experiments on trees growing under conditions of high rainfall and often on poorly drained soils. The work was designed to determine whether *P. cinnamomi* is the primary cause of the decline or collapse disease, and if so, whether a resistant root stock can be found or methods of planting or site selection be used to eliminate the disease or hold it to a minimum.

HISTORY OF THE DISEASE

Avocado root rot caused by *Phytophthora cinnamomi* was first reported from Puerto Rico in 1929 by Tucker (10, 11). These first reports showed that conditions of waterlogging and *P. cinnamomi* had to be present to cause the disease. Wager (12) in 1940 reported the disease in California and expanded the experimental work demonstrating the connection between conditions on poor soil drainage and ability of the parasite to cause disease. Specific experimental evidence was presented showing that avocados grow-

ing in pots could be artificially infected with *P. cinnamomi* and disease symptoms produced if the pots were submerged for a period as short as 2 or 3 days, while no infection or disease symptoms would appear in trees given normal watering. In 1941 Wager (13) reported the disease from Western Transvaal and Natal in South Africa. Field observations stressed the appearance of the disease on trees growing in heavy clay soils or on poorly drained sites. Stevens and Piper (9) reported what was later determined to be the same disease from Florida in 1941. Wager (14) in 1942 amplified his earlier work demonstrating the relation between wet soil and the disease.

An open discussion on decline of avocado trees on wet soil was reported in the 1937 California Avocado Association Yearbook (1). It seems evident that the problem under discussion was the root disease caused by *Phytophthora cinnamomi*, then unknown in California. The suggestion that waterlogging was causing the decline was questioned when it was brought out that the avocado came originally from regions of high rainfall. Various reasons were advanced to support the argument that waterlogging caused the disease, among them that in the tropics the trees grow on well drained soils or that they are adapted to the local condition. A heavy loss observed in Mexico after an unusually wet season was mentioned and the suggestion made that sudden change could be disastrous. Decline was again discussed, by Rounds (8) in the 1939 Yearbook of the Association, and also, in the 1941 Yearbook (2) when Wager's work (12) was mentioned. In 1943 Klotz and Sokoloff (6) tentatively expressed the view that *P. cinnamomi* and other pathogenic fungi might attack the roots after they were predisposed by injury following waterlogging. Parker and Rounds (7) in 1943 showed the relation of soil moisture and drainage to decline of mature avocado trees and while citing work such as Wager's (14) in relation to soil moisture and decline did not mention the connection of *P. cinnamomi* with this decline. In 1944 Harvey (5) reported the results of a survey made in California by the Emergency Plant Disease Prevention Project to determine the incidence of *P. cinnamomi* in relation to avocado decline. He isolated *P. cinnamomi* from the roots of 164 out of 268 healthy appearing trees growing in close proximity to declining trees. Zentmyer, Klotz, and Miller in 1945 (17) presented experimental evidence that tends to show that avocados decline in flooded pots regardless of the presence or absence of *P. cinnamomi*. Zentmyer and Klotz in 1947 (15) stated that *P. cinnamomi* may be a primary factor in decline of avocado trees but later the same year (16) stated that poor drainage generally initiates the trouble. They further report that waterlogging and *P. cinnamomi* appear to be involved in decline and that trees in waterlogged soils will decline without the fungus being present but that the presence of *P. cinnamomi* will accelerate the decline.

The relationship between soil moisture, drainage and *P. cinnamomi* is recognized on other hosts. Crandall, Gravatt, and Milburn (4) showed

the relationship of the recession of the American chestnut and chinkapin with conditions of soil drainage and also report a number of nursery diseases of various hosts clearly linked with predisposing conditions of flooded soils. The relationship between high moisture conditions and parasitism by the genus *Phytophthora* in general is well known.

GEOGRAPHICAL DISTRIBUTION

Phytophthora cinnamomi is not listed in La Flora Fungosa Peruana by García Rada and Stevenson published in 1942, on avocado or other hosts. The writer has heard verbal accounts of epidemic losses of avocados from root rot in other regions of Peru which occurred some years ago and that could have been caused by *P. cinnamomi*. However, so far as has been determined, the disease is present only in the Tingo Maria region. There seems no reason to suppose, however, that it may not be of widespread occurrence on avocados wherever they are grown under environmental conditions favorable to the fungus.

SYMPTOMS OF THE DISEASE

In this region two distinct methods of attack have been observed on both seedling and mature trees. The first is characterized by appearance of chlorosis followed shortly by sudden wilting and death (Fig. 1). The second

FIG. 1.   Healthy appearing avocado tree on left contrasted with, right, avocado wilting as a result of canker at the collar.

is a slow decline of the trees characterized by die-back of the branches and often typical drought symptoms in the leaves. In small trees with slow decline symptoms, death usually follows during the same season but mature trees seem to partially recover during the dry season. Some trees under observation for three years, are still in little worse condition than when first observed.

Light brown girdling cankers in the cambium and phloem tissue at the collar region and immediately below are found on trees showing chlorosis and sudden wilting. The advancing margin of the infection extending into healthy tissue is irregular and wedge shaped. The surface of this infected area is black or darker brown than the surrounding healthy bark. Isolated islands of infection and dead or infected small rootlets may be present or absent. In contrast, trees showing slow decline, either completely lack the infection at the collar or it is present on one side or portion of the collar only. The small rootlets are dead or infected, and islands of infection may be present or absent on the lateral and main roots.

ISOLATION AND INOCULATION

During the wet season *Phytophthora cinnamomi* appeared in all of the tissue cultures from the active cankers in the collar region, in 97 per cent from the smaller rootlets, in 18 per cent from the larger roots, and in 9 per cent from the islands of infection on the larger roots when special techniques for Phytophthora isolation were used.[3] Isolation attempts during the dry season and isolation by routine methods have been uniformly unsuccessful.

Six one-year-old criollo avocados growing in boxes were used for a preliminary proof of pathogenicity following the first isolation of *Phytophthora cinnamomi* from a dying tree. Three were inoculated by placing a mycelial mat of the *Phytophthora* in the soil against the collar and three were left uninoculated as checks. The trees were not watered artificially but were left outside during the rainy season. The three inoculated trees died before the end of the wet season while the three check trees were still healthy at the end of the following dry season. *Phytophthora* was recovered from the dying inoculated trees.

A project was set up primarily to test the resistance, by inoculation, of the different races of avocado. In October, 1945, the Station received, through the U. S. Bureau of Plant Industry, Office of Foreign Plant Introduction, 100 seeds of the Gottfried variety (Mexican race), 39 seeds of the Family variety (an old Florida selection and probably from a cross of the West Indian and Mexican races), and 32 seeds of West Indian seedling selections. Eighty-two of the Gottfried variety, 11 of the Family, and 10 of the West Indian seeds germinated and produced healthy plants. These were used with 130 criollo avocados, 19 of which were reserved as uninoc-

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[3] These methods principally consisted of placing carefully selected sections in sterile water blanks for periods varying from 3 to 5 days with frequent changes of water and then lightly surface sterilizing before planting on cornmeal agar.

ulated checks in a separate bed. The plants were in well drained beds of friable clay-loam and the varieties were separated by drainage canals. The plants were inoculated in late January and early February by placing mycelial mats of *Phytophthora cinnamomi* against the collar of each tree. Two months later 80 per cent of all inoculated plants were dead or dying, and before the end of the dry season in September a 100 per cent loss had occurred.

Phytophthora cinnamomi was isolated from the dying plants. The un-inoculated check plants showed no symptoms of disease throughout the wet season and the following dry season. By January, 1947, they uniformily showed symptoms of root rot and quickly declined. *P. cinnamomi* was isolated from these plants.

EPIDEMIOLOGY

Tingo Maria is at an elevation of approximately 2200 feet on the Huallaga River, a tributary of the Amazon in the Peruvian montaña. The year is divided into rather distinct wet and dry seasons. Records since 1940 show an average rainfall for the 6 dry months of April through September of 43.25 inches, and of 91.66 inches for the 6 wet months of October through March. The driest month was July, 1943, with 1.2 inches, and the wettest month was December, 1945, with 25.8 inches. The wet season of 1940–41 had the most rainfall, a total of 106.7 inches for the 6-month period. The 1944–45 wet season had 78.6 inches and the 1945–46 wet season 99.1 inches. Soil pH of avocado sites ranges from 4.5 to 7.0.

Losses from root disease have been more noticeable either at the beginning or toward the end of the wet season, probably because symptoms are more in evidence at these times. The disease apparently appears at any time during the wet season and with the exception of trees parasitized during the wet season but dying after the onset of dry weather, is apparently inactive during the drier months.

Avocados are not grown in the region in large plantings. The majority of the small land owners have a few trees each to produce fruit for home use and limited local sale. The bulk of these trees are criollo seedlings. No losses of epidemic proportions in any given area have occurred. From time to time a loss of one or two isolated trees is observed. In almost every case observed of trees developing chlorosis and sudden wilting, healthy trees remain on closely adjacent sites. Individuals and small groups have died on poorly drained sites but healthy individuals remain on adjacent equally poorly drained sites. Likewise similar losses on well drained sites have been adjacent to apparently healthy trees. Examination of the root systems of these apparently healthy trees has disclosed that the roots and rootlets were generally in good condition, although some rootlets were dead and some isolated cankers were present. The direct cause of the wilting and death were girdling cankers at the collar. *Phytophthora cinnamomi* was isolated from the girdling cankers.

Only one instance of what might be called slow decline or die-back has been observed. This is in a grove of mature and semi-mature trees located on the grounds of the Experiment Station. This group presents in greater or less degree, the die-back and decline symptoms. Only a few have died and the condition of the remainder appears little worse now than 3 years ago. At least partial recovery seems to take place during each dry season. Partial excavation of the root systems has shown a surprising lack of living small rootlets. *Phytophthora cinnamomi* has been isolated from the dying rootlets on these trees. The trees are growing on a site which is well drained but with heavy clay soil.

During the current wet season symptoms of drought in the leaves and die-back appeared in beds of seedling avocados being grown for use as budding stocks in the same general area but on a loamy soil. Higher and better drained parts of the beds were at first unaffected. Examination of the seedlings with drought symptoms and die-back showed that almost all the small rootlets were dead or dying. No collar infections were found. *Phytophthora cinnamomi* was isolated from a high percentage of the dying small rootlets.

CONCLUSIONS

Studies of the disease made in this region, under environmental conditions which are probably normal for the avocado, perhaps give a clearer picture of the disease as a whole than studies made in regions where the tree has been introduced and is growing under abnormal conditions. Any controversial issues of the role of pathogen versus wet feet do not seem to be important where the tree is growing normally under rather wet conditions. The reported experiments which tend to show that *Phytophthora cinnamomi* is a pathogen only when the tree is subjected to conditions of poor drainage, actually probably only reproduce conditions which often occur in regions where the tree is in its normal environment.

Viewed in relation to the appearance of this disease in the Tingo Maria region the various views previously summarized do not present a controversial issue. Each presents a portion of the picture and the sum total seems merely to show that under certain conditions the disease is not a factor unless soil conditions approach those often normally present in the tropics during the wet season. Undoubtedly trees grown under dry conditions with root system developed for these conditions would suffer if the environmental conditions change toward the wet side. Likewise such trees would then be growing under conditions ideal for attack by *Phytophthora cinnamomi*.

Seen under local, normal conditions this disease does not appear different, except possibly in degree, from other root diseases caused by Phytophthoras. Like many such Phytophthora-induced diseases, conditions of high soil moisture are usually required before the fungus parasitizes its host. Whether these conditions constitute predisposing conditions by adversely

affecting the host or whether they only create the conditions necessary for growth of the fungus is beside the point. Probably, dependent upon the amount of shift of the environmental conditions away from the optimum for the host and toward that of the parasite, both occur. The writer personally feels that in this and other tropical and subtropical Phytophthora-induced root diseases the effect of the change toward wetter environment is more important to the fungus parasite than the host. Improvement of planting techniques and site selection with the objective of better soil drainage have given beneficial results in the case of this disease as well as others, when a susceptible host must be grown in the presence of a Phytophthora parasite.

In the face of this disease, widespread planting of avocados is not being encouraged in this zone. For such trees as are planted only the better drained sites on sandy or loose, friable soils are recommended. The uniform susceptibility of the avocado races thus far tested indicates that development or selection of a resistant root stock is not a likely possibility unless some resistant parent type exists. Search is still being made for such a type.

SUMMARY

Phytophthora cinnamomi, root rot of avocados, has been found in the Tingo Maria region on well and poorly drained soils, on light and heavy soils, and on mature and nursery age trees. In all cases examined, healthy trees were growing on the same sites and under similar conditions but without disease. Two manifestations of the same disease have been studied. Mature and nursery age trees which develop chlorosis and sudden wilting (what apparently is called quick decline in California) are infected at the collar region with girdling cankers. Slow decline and die-back on both mature and nursery age trees apparently are caused by infection and death of the smaller rootlets, with the main roots and collar generally free from infection. *Phytophthora cinnamomi* has been isolated from both types of infections. The pathogenicity of the strains isolated has been demonstrated by artificial inoculation on trees growing under normal wet season conditions, with symptoms of chlorosis and sudden wilting. The fungus has been recovered from inoculated trees following the appearance of the symptoms. Uninoculated check trees, growing under identical environmental conditions. have remained healthy.

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GLOEOSPORIUM DECAY IN GRAMINEAE[1]

RODERICK SPRAGUE[2]

In an earlier report (7), *Gloeosporium sp.* was mentioned as present on the roots of various grass and cereal hosts in North Dakota but, except for one instance, it had proved non-parasitic. Recently the writer (8) reported that several isolates of a carrot-red, mucose *Gloeosporium* caused seed rot of various plants in sterilized soil. On the basis of this work, further study was made with the *Gloeosporium* that produces carrot-red masses of conidia on potato-dextrose agar and also with a common strain that develops pink masses of conidia on the same medium.

HOST RANGE AND GEOGRAPHICAL DISTRIBUTION

This fungus has been isolated from 121 species of cereals and grasses and its host range on this family appears to be virtually unlimited. It would appear, therefore, to be unnecessary to list so lengthy an array of hosts in this article. All cereals and common species of grasses in North Dakota harbor the fungus.

This species has been isolated from field material collected in Minnesota, North Dakota, South Dakota, Nebraska, Wyoming, Montana, and eastern Washington, and it probably occurs over a far wider area.

PURE CULTURES

The fungus is readily isolated on potato-dextrose agar from water agar plates of water-washed roots and crowns. The isolates grow very slowly at first but eventually they cover test tube slants with yeasty masses of conidia. The isolates, which form pink spore masses, first produce pale cream-colored colonies. These soon become bright pink masses of conidia that eventually germinate to form black smooth or wrinkled carbonaceous colonies. Sometimes pycnidium-like stromata are formed. On sterilized sand plus 0.85 per cent bran and 0.5 per cent dextrose, the colonies are gray, cottony, and composed of true mycelium. Sometimes chlamydospores similar to those formed by *Fusarium oxysporum* (Schlecht.) em. Snyder and Hansen occur.

The isolates that produce carrot-red masses of conidia are possibly somewhat slower in forming black stromatic hyphae. There are a few isolates

that are intermediate in color between the pink and the carrot-red types while there are still other isolates that scarcely sporulate at all and resemble cultures of *Phoma*.

In an earlier article (7) the writer reported 919 pure cultures of *Gloeosporium* out of a total of 22,832 cultures of all fungi isolated up to that time. *Gloeosporium* thus constituted about 4 per cent of the total. In 1944, *Gloeosporium* averaged 6.7 per cent of the total while during the cool season of 1945, it averaged over 14 per cent of that year's isolates. During mid-January, 1946, 26 per cent of the isolates from dormant grasses were referable to *Gloeosporium* (8). In these January isolates of *Gloeosporium*, 96 were the pink strain while 52 were the carrot-red one. The pink strain usually has been the more common one, particularly during the summer months. Both strains, however, were isolated from necrotic roots during all months of the year. In 1947, a dry season, only 1 per cent of the isolates from the roots of Gramineae at Pullman, Wash., were *Gloeosporium*.

ARTIFICIAL INOCULATIONS

This species appears to be one that performs erratically under artificial inoculation conditions. Inoculum was increased on the sand-bran-dextrose formula under aseptic conditions in milk bottles and added one inch beneath the seed in electrically sterilized soil in $14 \times 20 \times 4$ inch flats in the greenhouse. The temperature was variable, ranging from $55°$ to $80°$ F. during the latter part of the winter of 1945–46.

The results of all recent inoculation trials with *Gloeosporium* are summarized in table 1. Percentage loss was calculated the same as in earlier trials (7). Seven tests with the pink isolates showed two parasitic isolates, two that were questionable, and three that were non-parasitic. Of the carrot-red isolates, five were positive, one questionable, and two negative. Of the five carrot-red isolates that were positive, however, only three were original field isolates while the other two were reisolates from one of the original positive cultures.

All of the results indicate that *Gloeosporium* is short lived under artificial greenhouse conditions. Apparently saprophytic contaminants (mostly bacteria) soon suppress it in these greenhouse trials. Before the fungus is suppressed, however, it sometimes causes complete pre-emergence death of the seed of some hosts such as blue grama or alfalfa. Plants with larger seeds such as corn, beans or pumpkins, had sufficient food reserve to permit the seedlings to survive and recover. In one case, however, even pumpkins were not only retarded but the seed rotted. Sometimes wheat had all the roots stubbed off by *Gloeosporium* so that the stunted culm and the attached brown, decayed seed were readily lifted from the flat.

As mentioned, infection is very erratic with the methods used and therefore no attempt was made to distinguish race differences. For this, it probably will be necessary to work out a technique similar to that used by Jaarsveld (3) with *Rhizoctonia solani*.

TABLE 1.—Results of inoculating sterilized soil at seeding time with sand-bran-dextrose cultures of Gloeosporium sp. in the greenhouse at Mandan, N. Dak., 1941-1946

Culture No.	Original Host	Color of Strain	Percentage loss due to rot on:																	
			Alfalfa	Beans, Field	Beets, Garden	Bluegrass, Ky.	Brome, Smooth	Cabbage, Chinese	Corn, Field	(Grama), Blue	(Grass), Pigeon	Hordeum brevisubulatum	Oats, Common	Proso, Turghai	Pumpkin, Pie	Sorghum, Amber	Tomato, Rosana	Wheat, Common	Wheatgrass, Standard Crested	Wheatgrass, Intermediate
53C-1	Brome, Smooth	Carrot-red				0	0			0			0					0	0	
56B-4	Brome, Smooth	Pink		50	29	0	tr			0			0					94	23	30
Check							0						0					0	0	
3701A-6	Elymus excelsus	Carrot-red	96					92					5					28	32	
49C-1	Grass, Pigeon	Pink				2	0		tr	40	78	29	0	68	1	7	20	0	0	
116A-1	Muhlenbergia japonica	Pink				0	0		0					0				0		
2613A-1	Timothy	Pink	100			50a			32	93			13	0		24		65	65	
2649A-5	Wheat, Common	Pink	0			0a			0	30			0	98		0		0	tr	
5B-1	Wheatgrass, Crested	Intermediate				0	0						0					0	0	
217A-4	Wheatgrass, Crested	Pink	0			0	0						0					0	0	
1740A-7	Wheatgrass, Crested	Carrot-red	100			0a				40			0	0		0		0	0	
2624A-1	Wheatgrass, Crested	Pink	100			5a			6	64			0	55		40		tr	10	
3200B-1	Wheatgrass, Intermediate	Carrot-red	0			0	0b		0	0	6	2	0							
3263A-1	Wheatgrass, Intermediate	Carrot-red	26	12	36	9		82	tr	8	88	25	tr	16	35	tr	50	4	14	0
3257A-2	Wildrye, Canada	Carrot-red	84	60	58	40		75	21	69	88	29	0	7	0	tr	0	4	10	4
3705A-1	Reisolation of 3257A-2	Carrot-red	100	60		35		100	tr	68	24	tr	4	96	85	14	40	31	30	28
3708A-4	Reisolation of 3257A-2	Carrot-red	60	40		54		82	tr	25			1	8	45	tr	tr	9	40	20

a Canada bluegrass.
b Bromus rigidus.

The results obtained indicate that both the pink and carrot-red strains are sometimes capable of causing seed rot but that the species is seldom actively parasitic. Isolates frequently were obtained from plants growing in nitrogen-starved soil, in crowded volunteer stands, or from plants growing in cool wet soil. The fungus apparently is a competitor with the plant roots for soil nutrients and its action in general is probably detrimental. The fungus needs study to determine its straw-rotting ability and to determine if its injury is possibly the result of enzymatic action.

MORPHOLOGY

Conidia of both the pink and carrot-red strains appear to be identical (Fig. 1) although spores of the pink form tend to "blow up" to a larger size in old, semi-vacuolated spores. Younger but mature spores of both types are hyaline and non-guttulate but they soon become 1–4 guttulate and somewhat distended. The spores average $4–9 \times 1.7–3.0 \mu$ but some
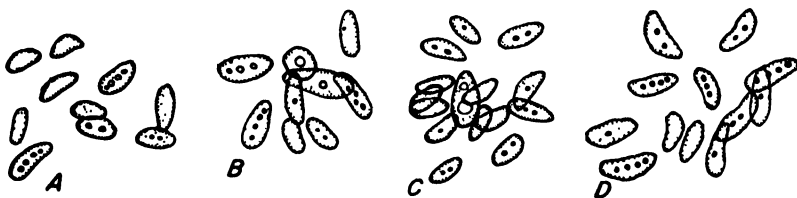

FIG. 1. Conidia of *Gloeosporium bolleyi* from pure cultures on potato-dextrose agar. *A.* from culture 3679 B–5, pink strain, isolated from *Hordeum brevisubulatum*, under snow, Mandan, N. Dak., Jan. 17, 1946. *B.* from culture 49 A–2, pink strain, isolated from *Agropyron cristatum*, Huron, South Dakota. *C.* from culture 5 B–1, intermediate strain, from *A. cristatum*, Mandan, N. Dak. *D.* from culture 3703 B–3, carrot-red strain, from *Elymus canadensis*, Mandan, N. Dak. (All × 1000.)

are as wide as 4μ; and extremely swollen or distorted ones average considerably larger.

Scattered among some of the colonies of conidia are chlorinous chlamydospores with the inner wall (endospore) somewhat reticulate. The hyphae formed from germinating spores are variable but usually are compacted to a carbonaceous, almost amorphous mass in older cultures. Bodies formed at the edge of older cultures appear to be stromatic.

TAXONOMY

Many workers have seen this *Gloeosporium* on Gramineae but it has frequently been confused with *Colletotrichum graminicola* (Ces.) G. W. Wils. The spores of the latter as given by Manns (*C. cereale* Manns) (6) are spindle-form to boat shaped, $18–26 \times 3–4 \mu$. In addition *C. graminicola,* according to Manns, produced steel gray to dark gray, compact downy growth and sporulation was not plentiful. The *Gloeosporium* under study has spores $4–9 \times 1.7–4.0 \mu$ and typically produces primary mucose colonies very different from those of *C. graminicola*. Because of the absence of setae, it belongs in the genus *Gloeosporium*.

Colletotrichum graminicola was reported on cereals in North Dakota by Bolley (1) but an illustration (by Milbrath, Fig. 21, *a*) in that article indicates that the North Dakota workers were probably dealing, in part at least, with the same *Gloeosporium sp.* that is the subject of this article. Böning and Wallner (2) describe a seedling blight, wilt, and footrot due to *C. graminicola* in Germany, while Sanford (5) found that this species caused a root rot in oats near Edmonton, Alberta. Winter (9) found the same type of fungus on wheat, barley, rye, and oats in Germany. All of these workers appear to have been dealing with the true *Colletotrichum* and not *Gloeosporium*.

There are two species of *Gloeosporium* described on Gramineae, both by Rostrup from Denmark (4). *G. graminum* on *Lolium multiflorum* has spores $11–14 \times 4–6\ \mu$ while *G. dactylidis* on *Dactylis glomerata* has small spores, $5 \times 1\ \mu$. Neither is similar to our fungus, which appears to be undescribed in spite of its prevalence. Since the most obvious name (*graminum*) has been used and since the host range and geographical distribution are so extensive, it appears desirable to name this species for an individual. We therefore name this for Henry L. Bolley, who noted the fungus early in the rootrot work and who pioneered in this subject. The fungus is described as follows:

Gloeosporium bolleyi sp. nov.

Acervulis (pseudopionnotes) in cultura copiosis, mucosis primo incarnatis v. aurantio-rubris demum nigris, carbonaceis; conidiis numerosis in cultura, hyalinis, subphaseoliformi-bus v. oblongis, eguttulatis v. demum guttalatis, $4–9 \times 1.7–4.0\ \mu$, plerumque $5–8 \times 1.9–2.5\ \mu$.

Hab. in rhizoideis languidis Graminearum, North Dakota, South Dakota, Nebraska, Wyoming, Montana, Minnesota et Washington. Typus est cultura 4180B–4, ex *Tritico aestivo*, Mandan, North Dakota.

The description is based on pure cultures of the fungus. The culture used as the type is a typical pink strain isolated from common wheat in the rotation plots at the Northern Great Plains Field Station, Mandan, N. Dak. Dried sub-cultures of the type have been preserved and filed in several herbaria.

SUMMARY

Gloeosporium bolleyi Sprague is common on the roots of many species of grasses and cereals in the western United States. Under artificial inoculation conditions in the greenhouse it sometimes causes seed rot and root necrosis of small grains and grasses.

The writer is again indebted to Miss Edith Cash for checking the Latin description and to Dr. A. G. Johnson for editing the manuscript.

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PHYSIOLOGICAL EXHAUSTION OF STRAWBERRY PLANTS AS A FACTOR IN WINTER KILLING

E. O. MADER AND A. W. FELDMAN

Mulching strawberry plants after they have been subject to "hardening" temperatures has reduced winter killing in Minnesota. Plants were injured when hardening temperature fell below $-6°$ C. and were killed at temperatures of $-12°$ C. and lower (2). Freezing and thawing temperatures, especially those ranging from $-6°$ C. to $6°$ C., occur during the fall and spring when strawberry plants are not mulched and occur frequently under the ice mulch cover during winter and spring. The question arises as to whether such fluctuating temperatures bring about physiological changes in the plants to make them more subject to winter injury or to predispose them to soil inhabiting pathogens.

EXPERIMENTAL MATERIALS AND METHODS

Field-grown, current-season plants of the variety Senator Dunlap obtained from Allens Nursery, Salisbury, Md., were used. These plants were obtained on two different dates, October 21 (Series 1) and November 15, 1946 (Series 2). The root systems for plants of both series were well developed, but plants of series 2 had more leaves and more fully formed crowns than plants of series 1.

Although the plants were free of disease so far as could be ascertained, undoubtedly some of them had incipient infections by root-rotting fungi. They also carried with them on their roots several organisms not necessarily pathogenic to them. In order to assure the presence of soil inhabiting pathogens, all plants of series 1 and part of those of series 2 were inoculated with isolates of *Fusarium* pathogenic to strawberry plants. The Fusarium isolates were grown on a sand-cornmeal medium. The plant roots were immersed in a suspension of these fungus cultures and were wrapped in moist sphagnum moss prior to storage. The plants were examined periodically throughout the storage period and the sphagnum wrappers were kept moist. Plants were stored at a constant temperature ($3°$ C.) or at a fluctuating temperature ($3°$ C. alternating with $-3°$ C.) for 12, 14, or 24 days (Table 1).

After each respective storage period, five plants were selected at random for analyses of total sugars (reducing and non-reducing) and starch. Triplicate determinations were made on each group from 2-gram, dry-weight samples. The Hagedorn-Jenson method for sugar determination was used (3). The remaining plants were potted in sterilized soil and placed in a greenhouse at $16.5°$ C., where they had 14 hours of light per day to insure the longday effect on growth.

TABLE 1.—*Storage conditions for strawberry plants*

Series and lot	Number of days in storage at									Total number of days in storage
	3° C.	– 3° C.	3° C.	– 3° C.	3° C.	– 3° C.	3° C.	– 3° C.	3° C	
1, A	14	.. ..	...	.....	. .	.	.....	....	.....	14
1, B	4	6	4	.....	.	.		.	....	14
2, A	12	..	....	.	.	.		.	.	12
2, B	24	....	.	. .	..	. .		.		24
2, C	......	3	3	4	2	..			.	12
2, D	....	3	3	4	2	3	3	3	3	24

ANALYSES, RESULT, AND DISCUSSION

There was more sugar in the roots and tops of plants subjected to alternate freezing and thawing during 12 and 14 days of storage than in plants stored for the same times at a constant temperature of 3° C. (Table 1). After 24 days in storage, sugars had increased slightly in the roots, but decreased slightly in the tops of plants held at a constant temperature of 3° C.; but the sugars in the entire plants were approximately the same as they had been after 12 days in storage (Table 2, series 2, B and A). At fluctuating temperatures there was a lower sugar content in both roots and tops of plants stored for twenty-four days than in plants stored for twelve days (Table 2, series 2, D and C).

Starch content decreased very rapidly in the roots and rather slowly

TABLE 2.—*Sugar and starch determinations for strawberry plants stored at a constant temperature or at fluctuating temperatures*

Determinations	Stored at 3° C.		Stored at 3° C. alternating with – 3° C.	
	Milligrams of sugar or starch in one gram dry tissue			
	Roots	Tops	Roots	Tops
	Series 1, A[a]		*Series 1, B*[a]	
Total sugars	60.0	30.0	70.0	43.0
Reducing sugar	50.7	25.0	55.0	36.3
Nonreducing sugar	9.3	5.0	15.0	6.7
Starch as glucose	22.5	31.3	17.5	27.5
	Series 2, A		*Series 2, C*	
Total sugars	98.0	40.0	115.0	48.0
Reducing sugar	76.0	40.0	91.5	41.3
Nonreducing sugar	22.0	0.0	23.5	6.7
Starch as glucose	45.0	50.0	40.0	45.0
	Series 2, B		*Series 2, D*	
Total sugars	102.5	35.3	98.0	30.0
Reducing sugar	78.5	35.3	76.5	29.0
Nonreducing sugar	24.0	0.0	21.5	1.0
Starch as glucose	25.0	48.1	21.9	37.5

[a] Series 1, A and B were stored for 14 days; Series 2, A and C for 12 days; Series 2, B and D for 24 days.

in the tops as the storage period lengthened. There was less starch in the plants stored at fluctuating temperatures than in those held at constant temperatures (Table 2).

Plants of series 2, which had a relatively vigorous crown development when placed in storage, contained more total sugar and starch in their roots and tops after twenty-four days of storage than those of series 1 that were stored only fourteen days.

New roots and shoots were observed on plants removed from storage and placed in the greenhouse for twelve days. They appeared on a higher percentage of plants stored at fluctuating temperatures than on plants stored at a constant temperature, regardless of the length of the storage (Table 3). Cold treatment apparently had a stimulatory effect on the plants, and re-

TABLE 3.—*Growth responses of strawberry plants in the greenhouse after different storage temperature treatments*

Series and storage condition	Total number of plants[a]	Percentage of plants in each growth response class after							
		12 days				36 days			
		Dead	Inactive	Weak	Vigorous	Dead	Inactive	Weak	Vigorous
Series 1 (14-day storage)									
A. 3° C.	30, Inoc.	0	60	40	0	17	0	40	43
B. 3° C. alternating with −3° C.	30, Inoc.	10	33	57	0	60	0	33	7
Series 2 (12-day storage)									
A. 3° C.	10	0	90	10	0	0	0	20	80
A. 3° C.	20, Inoc.	0	70	30	0	10	0	20	70
C. 3° C. alternating with −3° C.	10	0	30	60	10	30	0	40	30
C. 3° C. alternating with −3° C.	20, Inoc.	5	20	75	0	40	0	35	25
Series 2 (24-day storage)									
B. 3° C.	10	0	80	20	0	10	0	20	70
B. 3° C.	20, Inoc.	0	60	40	0	15	0	20	65
D. 3° C. alternating with −3° C.	10	0	30	70	0	50	0	40	10
D. 3° C. alternating with −3° C.	20, Inoc.	10	15	75	0	60	0	30	10

[a] Inoc. signifies that the plants were inoculated with Fusarium isolates.

sulted in an earlier growth response. This growth response did not result in more vigorous plants, because, after thirty-six days in the greenhouse, vigorous plants were more numerous in the lots stored at constant temperature. The higher sugar content in roots and tops of the plants stored at the alternating temperatures can be considered responsible for the earlier or quicker growth response. The depleted carbohydrate reserve however was not sufficient for subsequent growth and establishment of the plants, hence, many of them remained weak or died.

A similar growth response was observed after cold treatment with chrysanthemum cuttings by Mader and Phillips.[2] These workers subjected

[2] Mader, E. O., and Kyle Phillips. Induced root formation of chrysanthemum cutting by low temperatures. Not published.

chrysanthemum cuttings to temperatures ranging from 0.5 to 1.5° C. and found that such an exposure (6–21 days depending on varieties used) was equal in its effect to the treatment of cuttings with 50 p.p.m. indole butyric acid solution. Furthermore, such cuttings treated at low temperature (and also cuttings treated with indole butyric acid) contained a higher percentage of total sugars and less starch than nontreated cuttings. For any given variety used there was an optimum range within the low temperature treatment that gave not only an earlier rooting but also allowed for a normal development of the tops. When this range was overstepped, the time necessary for rooting was decreased but at the expense of shoot or top development, so that either a weak plant or death often resulted.

Strawberry plants stored at constant temperature (3° C.) survived better than those stored at fluctuating temperatures (3° C. alternating with – 3° C.). Sixty per cent of the plants in series 1, B, were dead 36 days after removal from storage at fluctuating temperature as compared with 17 per cent in series 1, A which had been stored at constant temperature (Table 3). Comparable losses occurred in series 2, but because plants of series 2 had greater vigor than those of series 1 before storage, the losses did not reach 60 per cent and 17 per cent, respectively, unless the storage period was 24 days.

Inoculation with isolates of *Fusarium* known to be pathogenic to strawberries appeared to increase losses only slightly (Table 2). Approximately 10 per cent more of the inoculated plants died than noninoculated plants. To what extent these losses are due to a weakening of the plants which results in physiological exhaustion and finally death, or due to the action of soil-inhabiting pathogens, or to the interaction of these factors remains to be proven.

Similar results were obtained by Eide and Voorhees,[3] who subjected inoculated and noninoculated plants (inoculated with isolates of *Fusarium* pathogenic to strawberries) to temperatures of – 3° C. There were numerous lesions on the roots of inoculated plants and they wilted after they were removed from the low temperatures and placed in the greenhouse. The noninoculated plants also subjected to freezing treatments did not differ greatly from the noninoculated plants left in the greenhouse for comparison. Plants inoculated but not subjected to freezing had few lesions on the roots and there was no loss of vigor. These results are in agreement with those found by the present writers, except that in our experiments noninoculated plants subjected to alternate freezing and thawing were greatly impaired in vigor. It is possible that presence of incipient infections or activity of saprophytes on the roots of these field grown plants may account for this difference.

It is a known fact that when temperatures are below 4.5° C., carbohydrates are transformed in many plants (5, 6). This is followed by marked acceleration in respiration, particularly when temperatures drop to about

[3] Eide, Carl J., and R. K. Voorhees. Unpublished data. University of Minnesota.

0° C. (1, 4). Under the conditions of these tests carbohydrate conversion and apparent increased respiration took place in the strawberry plants. This conversion was also apparent in the plants stored at the constant temperature of 3° C. (a storage temperature of 5° C. was not available during the time these were run) but was more pronounced in those kept at the alternating temperatures during storage. The decrease of starch and in particular the decrease of sugar in the plants held for 24 days at the alternating temperatures suggest that increased respiration under these conditions must have been responsible for the physiological exhaustion and subsequent death of the plants.

These experiments indicate that strawberry plants subjected to alternating freezing and thawing temperatures will undergo a physiological weakening or exhaustion leading to ultimate death. Soil inhabiting plant pathogens are not necessary to bring about the death of such plants, but their presence will be a contributing factor in the killing of the plants. It appears that whatever means and methods are taken to maintain the carbohydrate reserve in the strawberry plants will reduce the probability of losses due to winter-killing.

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THE CARBOHYDRATE METABOLISM OF GERMINATING PHYMATOTRICHUM SCLEROTIA WITH SPECIAL REFERENCE TO GLYCOGEN [1]

DAVID R. ERGLE [2]

Previous studies (3, 4) of the root rot fungus, *Phymatotrichum omnivorum,* provided the basis for the work here reported. In those studies it was observed that sclerotia, formed in soil cultures of the fungus, continued to accumulate substantial amounts of polysaccharides during a 65-day developmental period. The major portion of the polysaccharides was identified as free and bound glycogen which together comprised approximately 37 per cent of the dry-weight of mature sclerotia.

Conceivably, these reserves play a vital role in the perpetuation of the fungus which, in the sclerotial stage, is known to have remained viable for several years (8). It follows that information on the mechanism of mobilization and utilization of these reserves would be of interest as a contribution to the basic knowledge of the fungus. The obtaining of such information was the primary objective in the current investigation.

MATERIALS AND METHODS

Production, Germination, and Preparation of Sclerotia for Analysis

Using navy beans as the principal organic nutrient, sclerotia were produced by culturing *Phymatotrichum omnivorum* in Houston Black clay (2). These were separated from the soil by careful washing and screening, and the surface water was removed by blotting prior to weighing. Sclerotia thus obtained from three lots of 30- and 60-day-old cultures of *P. omnivorum* were used separately in a series of germination experiments. Samples from each of the lots were retained for chemical analysis so as to establish the composition of sclerotia at the start of each experiment.

Three-gram portions of sclerotia were weighed into sterile Petri dishes and surface sterilized by agitating with a 0.5 per cent solution of sodium hypochlorite for one minute. The solution was decanted and the sclerotia were washed once with sterile tap water and again with a sterile, dilute inorganic nutrient solution (7) pH 6.5. About 2 ml. of the latter was left to cover the bottom of the dishes without submerging the sclerotia. The Petri dishes, 50 for each of the lots of 30- and 60-day sclerotia, were placed in an incubator maintained at 28° –30° C., and the sclerotia were allowed to germinate and the mycelium to develop for periods of 3, 6, 9, and 12 days. On the 6th day an additional 2 ml. of the nutrient solution was added to replace water losses and to maintain the pH at or near 6.5.

At the end of each of the allotted periods of incubation the sclerotia and mycelium of 6 Petri dishes were combined into a single sample, killed in toluene vapor, and dried in a mechanical convection oven at 70° C. for 18 hours. After weighing for the determination of the dry weight, the material was finely ground in a semi-micro Wiley mill preparatory to analysis. The contents of 4 additional Petri dishes, two for each analysis, were used for the determination of amylolytic activity.

Chemical Methods

Amylolytic Activity.—The determination was made according to Spoehr's (6) procedure in which the increase in the reducing power of the reaction mixture (2 gm. soluble starch, 5 ml. phosphate solution, fresh sclerotia and mycelium from 2 Petri dishes, and water to make 250 ml.) was measured after incubating for 24 hours at 30° C. The results were expressed as milligrams of glucose per gram of oven-dry tissue.

Sugars.—The sugars were extracted with boiling 80 per cent ethanol in a Soxhlet unit and the reducing sugars were determined by the method of Wildman and Hansen (11). Nonreducing sugars (4) were determined after hydrolysis with 4 per cent hydrochloric acid (by weight) for 1.25 hours in an autoclave at 15 lb. pressure.

Mannitol.—The presence of mannitol in the sclerotia and mycelium was established by isolation in a pure form from a 95 per cent ethanol extract and the preparation of the hexaacetyl derivative, the melting point of which agreed with that recorded in the literature (5). Using the method of Todd *et al.* (10) mannitol was quantitatively determined in an aliquot of the aqueous solution used for the determination of sugars.

Free Glycogen.—The oven-dried, insoluble residue from the extraction of sugars was weighed and the free glycogen was extracted from duplicate 0.5-gm. samples with 10-ml. portions of hot water until a test for glycogen (a reddish brown color with dilute iodine solution) in the extract was negative. To the combined extracts of each sample containing 5 ml. of a citrate buffer solution at pH 5.0, there was added 0.2 gm. Takadiastase,[3] a few drops of toluene to insure sterility, and water to make a total of 50 ml. The digests, contained in tightly stoppered flasks, were incubated at 32° C. for 48 hours. The reducing sugars resulting from hydrolysis were determined and multiplied by 0.9 to convert to the glycogen equivalent.

Bound Glycogen.—The wet residue from one of the duplicate samples extracted for free glycogen was suspended in 5 ml. of 35 per cent aqueous potassium hydroxide and heated for 1 hr. at 15 lb. pressure. After removal of the insoluble cell-wall materials by centrifugation, the glycogen was precipitated from the cold alkaline solution by the addition of 3 volumes of 95 per cent ethanol and acidulation with concentrated hydrochloric acid. The precipitated glycogen was next washed with ethanol, dried, dissolved in water, and determined as in the case of free glycogen.

[3] The Takadiastase was a special, undiluted preparation of Parke, Davis, and Co.

Hemicellulose.—The remaining duplicate from the extraction of free glycogen was hydrolyzed with 4 per cent hydrochloric acid and the resulting sugars were determined and multiplied by the factor 0.9. The value thus obtained represented the sum of (a) bound glycogen and (b) hemicellulose. The latter was calculated by subtracting the value of (a) from that of (a) + (b).

Cellulose and Suberin.—These constituents were separately determined on hemicellulose-free samples of sclerotia and mycelium by methods described in detail elsewhere (4).

Total Carbohydrates.—Calculated as the sum of the determined carbohydrate fractions.
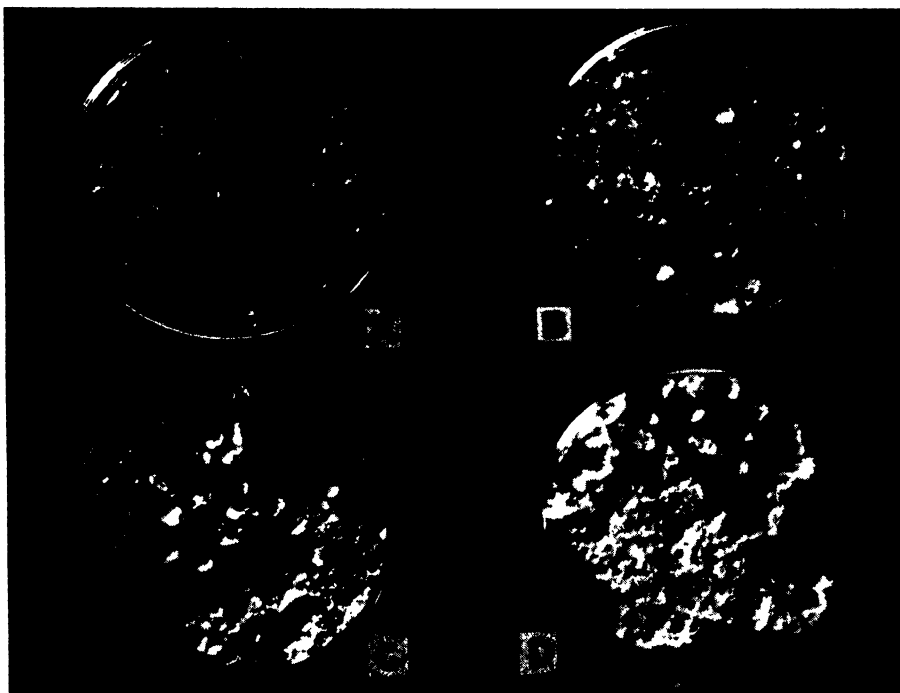
FIG.1. Dormant sclerotia (A), and those showing mycelial growth after incubating for periods of 3 (B), 6 (C), and 9 (D) days.

The chemical data are reported (a) as percentage of dry weight after the respective periods of germination and (b) as grams of constituents in the sclerotia and mycelium resulting from the initial 18 grams of fresh sclerotia. Each value is the average obtained from germination experiments that were replicated three times at three different dates.

RESULTS

Germination

Approximately 48 hours from the start of incubation, the initiation of germination processes was evidenced by the emergence of small, white tufts

of mycelium from the sclerotia.   As illustrated in figure 1 the density of mycelial growth increased with time except that there was no visible difference after 9 days.   Respiration, as measured by loss in dry weight, was high during the initial stage of germination, but after the 9th day there was little detectable change.   The two lots of sclerotia, though differing in age and in chemical composition, were remarkably similar in this respect. The 12-day losses in dry matter by 30- and 60-day sclerotia were 16.79 and 16.40 per cent of their original dry weights respectively, of which more than half of these losses occurred during the first 3 days of germination.

Changes in Composition of Germinating Sclerotia

The changes in the composition of germinating sclerotia as a percentage of the successive dry weights are given in table 1.   The behavior of the

TABLE 1.—*Chemical changes during the germination of sclerotia and growth of mycelium.   Data reported as percentage of dry weight*

Constituent	Sclerotia before germination	Sclerotia plus mycelium after germination			
		Days			
		3	6	9	12
		Sclerotia from 30-day-old cultures			
Mannitol	5.42	7.11	7.61	7.89	8.46
Reducing sugars	0.14	1.24	2.60	3.15	2.86
Nonreducing sugars	1.62	1.37	0.00	0.00	0.00
Free glycogen	10.57	2.64	1.94	1.32	2.56
Bound glycogen	26.35	26.71	20.21	18.41	17.83
Hemicellulose	9.90	10.40	11.46	12.27	10.81
Cellulose	0.60	0.68	0.65	0.91	0.90
Total carbohydrates	54.60	50.15	44.47	43.95	43.42
Suberin	2.88	3.84	4.69	4.32	5.01
		Sclerotia from 60-day-old cultures			
Mannitol	3.90	4.85	4.74	5.04	4.44
Reducing sugars	0.80	0.52	0.02	0.94	2.00
Nonreducing sugars	0.73	0.25	0.26	0.00	0.00
Free glycogen	4.07	2.03	1.67	1.79	1.31
Bound glycogen	30.85	24.34	21.82	17.94	16.39
Hemicellulose	12.23	16.02	16.63	16.37	14.83
Cellulose	0.68	0.86	0.88	1.05	1.12
Total carbohydrates	53.26	48.87	46.02	43.13	40.09
Suberin	2.74	3.81	4.87	5.31	5.20

concentrations of water-soluble and water-insoluble carbohydrate constituents as germination and mycelial development progressed is shown in figure 2.   The actual amounts of the various carbohydrate fractions involved in the germination are given in table 2 as grams of constituents resulting from the initial 18 grams of fresh sclerotia.

The germination of 30-day sclerotia for a period of 12 days resulted in actual losses of nonreducing sugars, free glycogen, bound glycogen, and hemicellulose (Table 2).   Gains were found in mannitol, reducing

sugars, cellulose, and suberin. The results for 60-day sclerotia differed in the following respects: (a) there was a slight loss in mannitol and (b) a gain in hemicellulose. Considering the net changes in all of the carbohydrate fractions as a whole, i.e., total carbohydrates, it was found that 18 grams of sclerotia from 30- and 60-day-old cultures decreased in carbohydrate content by 1.22 and 1.24 grams, respectively.

The initial 3-day period was characterized by relatively large losses in the free and bound glycogen contents of sclerotia. During this time the

TABLE 2.—*Chemical changes during the germination of sclerotia and growth of mycelium. Data reported as grams of constituents per initial 18 grams of fresh sclerotia*

Constituent	Sclerotia before germination	Sclerotia plus mycelium after germination				Gain or loss during germination
		Days				
		3	6	9	12	
	Sclerotia from 30-day-old cultures					
Total dry weight	6.55	6.00	5.64	5.53	5.46	− 1.09
Mannitol	0.36	0.43	0.43	0.44	0.46	+ 0.10
Reducing sugars	0.01	0.07	0.15	0.17	0.16	+ 0.15
Nonreducing sugars	0.11	0.08	0.00	0.00	0.00	− 0.11
Free glycogen	0.69	0.16	0.11	0.07	0.14	− 0.55
Bound glycogen	1.73	1.60	1.14	1.02	0.97	− 0.76
Hemicellulose	0.65	0.62	0.65	0.68	0.59	− 0.06
Cellulose	0.04	0.04	0.04	0.05	0.05	+ 0.01
Total carbohydrates	3.59	3.00	2.52	2.43	2.37	− 1.22
Suberin	0.19	0.23	0.26	0.24	0.27	+ 0.08
Amylolytic activity[a]	53	60	71	82	95	+ 42
	Sclerotia from 60-day-old cultures					
Total dry weight	6.28	5.73	5.49	5.36	5.25	− 1.03
Mannitol	0.24	0.28	0.26	0.27	0.23	− 0.01
Reducing sugars	0.05	0.03	Trace	0.05	0.11	+ 0.06
Nonreducing sugars	0.05	0.01	0.01	0.00	0.00	− 0.05
Free glycogen	0.26	0.12	0.09	0.10	0.07	− 0.19
Bound glycogen	1.94	1.40	1.20	0.96	0.86	− 1.08
Hemicellulose	0.77	0.92	0.91	0.88	0.78	+ 0.01
Cellulose	0.04	0.05	0.05	0.06	0.06	+ 0.02
Total carbohydrates	3.35	2.81	2.52	2.32	2.11	− 1.24
Suberin	0.17	0.22	0.27	0.28	0.27	+ 0.10
Amylolytic activity[a]	80	147	183	198	217	+ 137

[a] Mgs. of glucose per gm. of dry material.

free glycogen content of germinating 30- and 60-day sclerotia decreased 77 and 50 per cent, respectively, of the amounts originally present; the bound glycogen content of the 30-day sclerotia remained practically unchanged; and that of the 60-day sclerotia decreased 28 per cent. The remaining 9 days of germination and mycelial growth resulted in further progressive decreases in both free and bound glycogen except that the free glycogen content of germinating 30-day sclerotia was increasing by the 12th day.

The nonreducing and reducing sugars were present in ungerminated

sclerotia in relatively small amounts, but the latter in germinating 30-day sclerotia tended to increase linearly with time while the nonreducing sugars disappeared by the 6th (30-day sclerotia) and 9th days (60-day sclerotia) and were not reformed during the remaining period of the experiment. In the germination of the 60-day sclerotia the reducing sugar content approached zero in the first 6 days but subsequently increased to show a net gain of 100 per cent by the end of the experiment.

The mannitol content of both lots of sclerotia before and during germination exceeded by far that of the true sugars. Whereas the quantity of mannitol in germinating 30-day sclerotia increased consistently with time
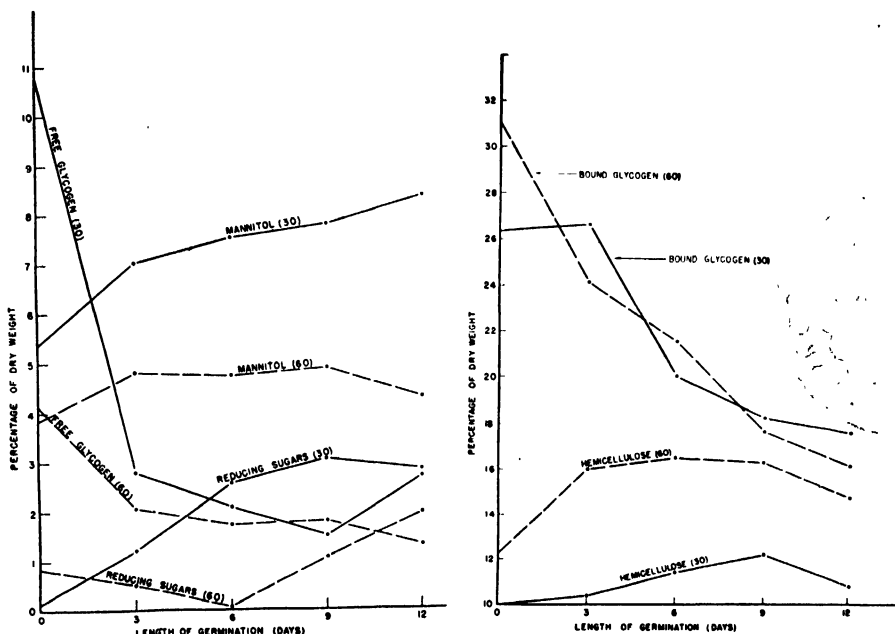
FIG. 2.   Left. Variations in concentration of the soluble carbohydrate fractions of germinating 30- and 60-day sclerotia.
   Right.   Variations in concentration of the insoluble carbohydrate fractions of germinating 30- and 60-day sclerotia.

and showed a net gain of 28 per cent, increases in the 60-day sclerotia were limited to a 9-day period after which there was a net loss of 4 per cent.

By the 9th day the quantity of hemicellulose in germinating 30 and 60-day sclerotia had increased 5 per cent and 14 per cent, respectively, but these increases were followed by decreases large enough to show a net loss of approximately 10 per cent in the former and a net gain of only 1 per cent in the latter. Cellulose and suberin were present in both ages of ungerminated sclerotia in approximately equal amounts, which increased 25 per cent and 42 per cent, respectively, during the germination of 30-day sclerotia, and 50 per cent and 59 per cent, respectively, in the 60-day sclerotia.

Amylolytic Activity

Amylolytic activity (Table 2) in the germinating sclerotia increased with time. The results on 30-day sclerotia showed a fairly constant rate of increase while those from 60-day sclerotia showed a diminution in the rate of increase. A more striking difference in both lots of sclerotia was found to be in their comparative amylolytic activities before and during germination. Before germination the ratio value of the activity in 60-day sclerotia to that in 30-day sclerotia was 1.6, but after germination started it increased to 2.4 and remained practically constant throughout the experiment.
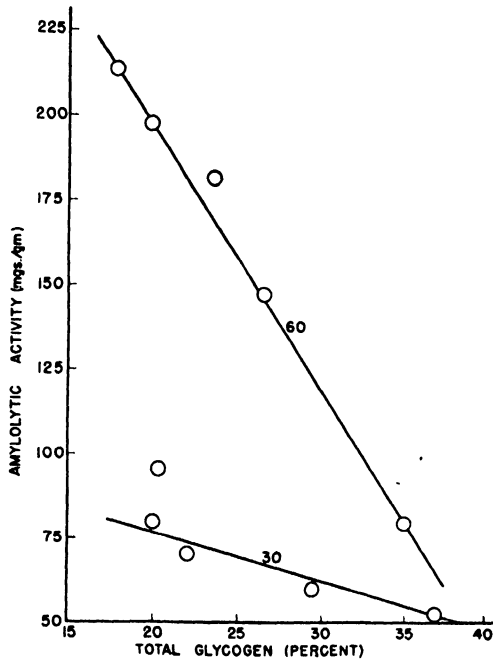
FIG. 3. Relation of total glycogen and amylolytic activity in germinating 30- and 60-day sclerotia.

Amylolytic activity in the germinating 60-day sclerotia was found to vary inversely with the concentration of total glycogen (free + bound), and a similar relation, though less consistent, was indicated during the germination of 30-day sclerotia (Fig. 3). In addition it was noted that a higher level of amylolytic activity (60-day sclerotia) was associated with higher levels of the insoluble carbohydrate metabolites.

DISCUSSION

The chemical and physiological processes found occurring in germinating sclerotia seem to reflect a pattern of metabolic sequences that is perhaps somewhat similar to that in germinating starchy seed. Like the latter, sclerotia of *Phymatotrichum omnivorum* are deficient in sugars and

the processes of respiration and synthesis depend upon the mobilization of sugars from other more abundant carbohydrates. The results of this study clearly show that glycogen, both free and bound, was the principal source of carbohydrate substrates with indications that nonreducing sugars and hemicellulose were sources of minor amounts. The mode of glycogen hydrolysis to glucose or to other metabolizable forms is suggested by the observed two-fold increase in amylolytic activity after germination started. The possibility that additional enzymes, glycogenase for example, were also functioning in the hydrolysis of glycogen is very likely. Also, Blank and Talley (1) showed that the mycelium contains additional carbohydrases that are capable of hydrolyzing a variety of simple and complex carbohydrates.

As reflected by the data, the next phase in the metabolic pattern was the utilization of the sugars mobilized from the various sources mentioned. Assuming that carbohydrates alone were used, the data show that respiration alone probably accounted for 89 per cent (30-day sclerotia) to 83 per cent (60-day sclerotia) of the observed losses. It is unlikely that either the fat (2.17 per cent of dry weight) or protein (18.98 per cent of dry weight) reserves (4) of sclerotia contributed materially to respiration as they are present in much smaller amounts than the carbohydrates. The remaining loss in carbohydrates may be logically attributed to the synthesis of noncarbohydrate constituents, such as suberin, by the mycelium. The utilization of some of the mobilized carbohydrates in the synthesis of mannitol, hemicellulose, and cellulose also was indicated as these constituents accumulated either throughout or during the greater part of the 12-day study. The accumulation of cellulose and suberin may be ascribed to cell wall formation by the mycelium of germinating sclerotia.

The results as a whole show that differences in composition and in age of sclerotia did not materially influence either the general course or the rate of carbohydrate metabolism. Before germination the 30-day sclerotia contained approximately twice the soluble carbohydrate (mannitol + sugars + free glycogen) content of the 60-day sclerotia while the latter were appreciably richer in insoluble reserves (bound glycogen + hemicellulose). Nevertheless carbohydrate utilization in the two lots of germinating sclerotia seemed to proceed at about equal rates, using the losses in dry matter and in carbohydrates as criteria. In this connection the finding of a higher amylolytic activity to be associated with higher levels of insoluble carbohydrate reserves might have been a factor in the equalization of the metabolic rates.

Since this study has shown that the glycogen reserves of sclerotia are the chief carbon sources during germination, the observed termination of mycelial growth after 9 days poses the question of possible glycogen depletion as a factor in limiting further growth. The data on the metabolism of glycogen are of little value in this respect as they reflect not only the

unused glycogen in the sclerotia residues but also that transported to or resynthesized in the mycelium. However, evidence of the presence of adequate amounts of glycogen in the sclerotia residues for further growth was obtained when it was found that the 12-day residues could be germinated a second time after the previous mycelial growth had been removed. In other laboratory studies Taubenhaus and Ezekiel (9) also showed that Phymatotrichum sclerotia under certain conditions may be germinated as many as five times. Thus it is apparent that the glycogen reserves of sclerotia are not readily exhausted during germination, and that the cessation in mycelial growth found in this study must be attributed to other causes.

SUMMARY

Sclerotia from 30- and 60-day-old soil cultures of *Phymatotrichum omnivorum* were allowed to germinate and grow on a sterile, inorganic nutrient substrate for periods of 3, 6, 9, and 12 days and the metabolism of their carbohydrate reserves was studied. The principal results may be summarized as follows:

1. The course and the rate of carbohydrate metabolism in the two ages of germinating sclerotia were essentially the same notwithstanding certain differences in the initial composition. Germination of 18 grams each of 30- and 60-day sclerotia resulted in losses of 1.09 and 1.03 grams of dry matter, respectively, and in corresponding losses of 1.22 and 1.24 grams of carbohydrates.

2. The initial 3-day period was characterized by intense metabolic activity as reflected in the loss during that time of approximately half of the total amount of carbohydrates used in the 12-day experiment.

3. Germination resulted in substantial net losses of nonreducing sugars, free and bound glycogen, and some hemicellulose (the latter during the 9–12 day period). Reducing sugars, mannitol (0–9 day period), cellulose, and suberin showed increases.

4. The glycogen reserves (free and bound) of sclerotia were the chief source of substrates for respiration and synthesis. The former process accounted for 83–89 per cent of the observed loss in total carbohydrates.

5. Glycogen mobilization was associated with a relatively high level of amylolytic activity, which tended to vary inversely to the concentration of total glycogen in the germinating sclerotia.

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PHYTOPATHOLOGICAL NOTES

Rootstock Susceptibility to Armillaria mellea.—The Santa Clara Valley is one of the principal deciduous fruit areas of California, devoted mainly to apricots, pears, and prunes. Pears on French pear roots are sufficiently resistant to Armillaria root rot to be excluded from this report. It may be mentioned, however, in passing, that pear trees on quince roots may be killed by this fungus. Apricot and prune orchards, on the other hand, have already sustained a loss of more than 10 per cent of the trees according to a survey[1] of 65 properties selected at random.

The most effective chemical treatment of the soil thus far, the injection of carbon disulfide, has been, on the whole, disappointing in this area. Unpublished work of the first and last authors indicates that this is partly due to failure of the chemical to penetrate to the lower depths (5 to 10 feet) in the rather heavy moist soils.

The alternative most commonly resorted to in Armillaria-infested areas of this valley is the planting of Persian walnut (*Juglans regia*) on Northern California Black walnut (*J. hindsii*) roots which seem to be adequately resistant to *Armillaria*. However, the disorder known as black line of walnut[2] is sufficiently prevalent in the area to raise some doubt as to the success of such plantings.

It seems to be obvious, at any rate, that an Armillaria-resistant rootstock for apricot and prune is needed in the Santa Clara Valley.

Eighteen lots of plum roots, mostly Myrobalan and Marianna types, were planted for trial at the University of California Deciduous Fruit Field Station at San Jose in 1928. These lots contained 37 to 40 trees and were planted about 36 inches apart in rows 48 inches apart. This crowded planting eventually necessitated pruning off all the lower branches to facilitate cultivation. Moreover, approximately half of each row was cut back heavily in the 2nd and 4th years from planting to simulate the usual procedure in topworking such trees in orchards. All of the trees were inoculated repeatedly by placing a handful of wood containing the fungus from pure culture adjacent to the crown. All the circumstances seem to have combined to provide a severe test of resistance. However, few of the trees were killed by *Armillaria* during the first five years. Trees which died were examined as soon afterward as feasible to determine the cause of death. Only those which seemed clearly to have been killed by *Armillaria* are so indicated in the summary of Table 1. The surviving trees were pulled in 1945, and the degree of root injury rated on a scale of 0 to 10, in which Class 0 was to represent trees with no infection, and Class 10 to indicate trees completely girdled at the crown and with most, or all, of the roots dead.

[1] Schneider, Henry, E. W. Bodine, and H. Earl Thomas. Occurrence of Armillaria root rot in Santa Clara Valley. U. S. Dept. Agr., Plant Disease Reptr. 29: 495–496. 1945.

[2] Smith, Ralph E. Diseases of fruits and nuts. California Agr. Ext. Circ. 120. 1941.

TABLE 1.—*Summary of results with plum planting at San Jose, California, for Armillaria resistance test after 17 years*

Selection	Number planted	No. surviving in 1945	No. killed by *Armillaria* in 9 years	Average degree of infection of survivors
Methley plum	40	37	0	7.6
Marianna, Tenn.	40	27	5	8.1
Do Calif.	40	32	2	7.1
Do 2624	39	35	0	6.7
Do 2623	40	29	3	7.1
Gypsie Myrobalan	40	18	4	6.2
Red Leaf Myrobalan	40	31	6	5.4
Purple Leaf Myrobalan	40	23	1	8.6
Myrobalan 29	38	30	0	7.5
Davis Myrobalan	40	27	1	8.5
Davis Myrobalan 7102	40	20	0	7.2
Goddard Myrobalan	40	7	3	8.7
Campus Myrobalan	40	30	0	7.4
Do	40	20	2	7.7
Do No. 3 . .	37	30	4	7.0
Black Damas D .	40	10	15	8.9
Do C .. . .:	40	6	7	9.2
St. Julien G	40	19	1	8.4

None of the 431 survivors fell into Class 0, and only 3 trees were good enough for Class 1. One of the 3 trees was sufficiently affected by crown gall to be discarded. Attempts are being made to build up new clones from the 2 remaining trees for further tests. One of these trees is from the lot designated as Marianna 2624 which in recent years has been in favor for orchard trials against *Armillaria*. A few root cuttings survived from this single tree, but, of course, at least a year or two will be required to increase these to any considerable number of trees. Although the Red Leaf Myrobalan

TABLE 2.—*Rating of some of the more resistant trees in the San Jose plot*

Botanical name	Common name	No. planted	No. of survivors	Average rating
Castanea dentata (Marsh.) Borkh.	American chestnut	40	27	0.74
Castanea sativa Mill.	Spanish chestnut	40	32	0.86
Diospyros kaki L.	Japanese persimmon	40	13[a]	0.85
D. lotus L.	Date-plum	40	36	0.88
D. virginiana L.	American persimmon	40	36	0.13
Ficus carica L.	Fig (Kadota)	40	39	0.10
Ficus carica L.	Black mission fig	39	28	0.25
Juglans hindsii Sarg.	Northern Calif. Black walnut	40	35	0.77
Pyrus betulaefolia Bunge.	Pear	39	15	2.93
Pyrus calleryana Decne.	Pear	40	24	1.90
Pyrus communis L.	Common cultivated pear	40	25	1.04
Pyrus malus L.	Cultivated apple "French crab" seedlings	40	31	1.10
Pyrus malus L.	Seedlings of Red Delicious apple	40	29	1.04

[a] Only one known to be killed by *Armillaria*.

had comparatively good average rating, survival was poor, and this lot contained no tree better than Class 2. The Myrobalan 29 of this test was not clearly better than some of the others, though trees under this number have been rather extensively planted in orchards. It now seems likely, however, that several different types of Myrobalan have been distributed under this number.

A number of other types of fruit and nut rootstocks were uprooted and examined in 1946 (Table 2). Some of these were planted 2 years later than the plum roots referred to in Table 1. Fig was least affected of all the trees examined, and the damage to this tree was slight. The best persimmon was hardly more affected. The row of Northern California Black walnut (*Juglans hindsii*) represented in Table 2, originated from seed of an isolated tree not likely to have been exposed to pollen of the susceptible Persian walnut. This row was rated about as expected on the basis of the oft-reported resistance of this species. It is probable that this lot of walnut and the figs would have rated even better had not meadow nematode and other agencies aided penetration by *Armillaria*. However, another row of black walnut under the same name and with no indication of hybridity, displayed little, if any, greater resistance than the plum roots which the black walnut is replacing in many areas infested by *Armillaria*. Some lots of apple scored surprisingly well in view of the fact that orchard trees are frequently killed by *Armillaria*. The best apples approached the pear in rating, the latter being seldom, if ever, killed by this fungus in orchards. The French pear (*Pyrus communis*) lived up to expectations and was definitely more resistant than the quince which is often used as a rootstock for some pear varieties. A comparatively small number of cherry rootstocks was available for observation, and these were not examined in detail. It was evident, however, that the Mazzard root was distinctly more resistant than Mahaleb cherry or the sour cherry.

The good score of several of the lots in Table 2 indicates that this test, though intentionally severe, was not too severe.

The wide differences in amount of infection between individual trees in single lots indicate that only single clones propagated by root or stem cuttings, when possible, should be used in such trials in the future.—HAROLD E. THOMAS, H. EARL THOMAS, CATHERINE ROBERTS, and AL AMSTUTZ, Division of Plant Pathology, University of California, Berkeley and San Jose.

The Microscopic Detection of Bacterial Infections in Plants by Means of Oblique Illumination.—F. C. Stewart observed the oozing of bacteria from the cut surface of infected plant tissues into a surrounding film of water as early as 1897.[1] This method of detecting bacterial infections was later used by Jones, Johnson, and Reddy[2] and by numerous others. As far

[1] Stewart, F. C. A bacterial disease of sweet corn. N. Y. Agr. Exp. Sta. Bul. 130. 1897.

[2] Jones, L. R., A. G. Johnson, and C. S. Reddy. Bacterial blight of barley. Jour. Agr. Res. [U.S.] 11: 625–643. 1917.

as we can determine direct lighting by means of the bright field condenser of the microscope has always been used when the microscope has been used to make this so-called "ooze test."

We have found that the use of oblique illumination to produce a dark field brings out the oozing bacteria much more clearly than does direct lighting. With oblique illumination the mass of oozing bacteria appear as a brilliant white column against a dark background (Fig. 1, A).

No special equipment is needed for oblique illumination. A microscope lamp producing light of fairly high intensity is satisfactory as a light source. The oblique illumination is produced by removing the condenser from the microscope and moving the concave mirror over to one side (Fig. 1, B). If the microscope does not have a concave mirror which can be
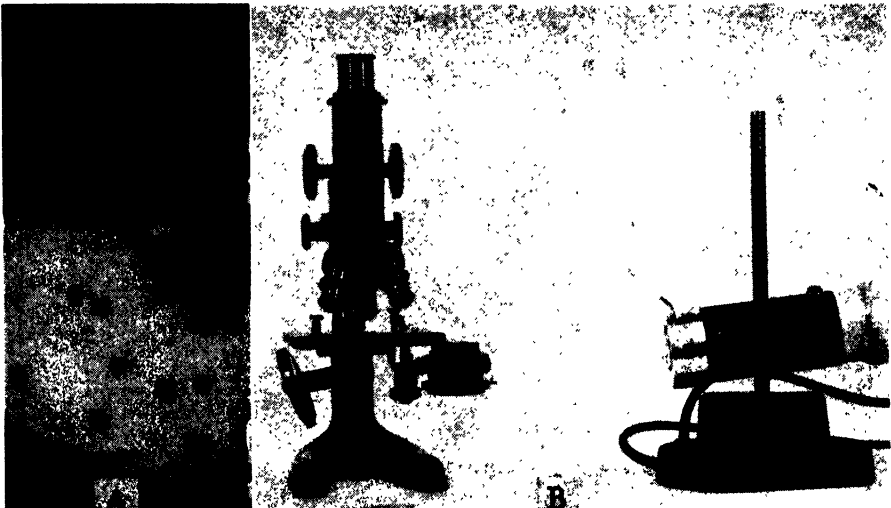
FIG. 1. A. Bright column of bacteria oozing from a necrotic spot in a cotton leaf. B. Arrangement of microscope and lamp for oblique illumination.

swung to one side the mirror may be attached to a ringstand and held in the oblique position.

The 16-mm. low power objective must be used in order to obtain a satisfactory dark field with this simple system of illumination.

In the case of bacterial leaf spots a cut through the middle of an infected spot is made with a razor blade, the cut tissue is mounted on a slide under a cover glass and a drop of clean water is added at the edge of the cover glass. With stems the cut end of a stem may be mounted in a drop of water without a cover glass or thick longitudinal sections may be mounted in water under a cover glass. The cover glass should not be touched after mounting if the bacteria are to remain near the point where they ooze from the cut end of the tissues.—T. E. RAWLINS and P. R. DESJARDINS, Division of Plant Pathology, University of California, Berkeley.

Rhizoctonia Rot of Tulips in the Pacific Northwest.[1]—A greenhouse planting of William Pitt tulips from Spokane, Washington, showed a top rot bulb infection in January, 1947. The injury was so severe that 7,000 out of 15,000 bulbs were either destroyed or injured to the extent that they did not produce marketable flowers. The infected bulbs were from two sources only, one said to be imported, the other one year from importation. Shipments of other varieties in the same greenhouse had no injury.

FIG. 1. Symptoms of *Rhizoctonia solani* injury on William Pitt tulips. (Photo by Sprague and MacLean.)

The symptoms somewhat resembled Botrytis fire but with certain distinct differences. The lesions on the bulbs were gray or gray-brown and somewhat dry (Fig. 1). The leaves from diseased bulbs were either killed or stunted, frequently adhered together, and bore the gray or gray-brown lesions. A white cottony mycelium grew on the necks of some of the bulbs. In severe cases the bulbs were almost rotted away. The roots of all except the most severely injured were still active, however.

Isolations were obtained from necrotic bulb parts on potato-dextrose agar, bean-pod agar, and prune agar. The mycelium developing from the

sections grew at only moderate speed, after a slow start. The light-brown mycelium had the characteristic right-angled branching and constrictions of *Rhizoctonia*. Hyphal strands measured from 6–8 μ in diameter (average 7 μ). The causal agent appears to be *Rhizoctonia solani* Kuehn.

In addition to the common Rhizoctonia mold, the usual species of *Penicillium* were present and also nematodes, mites, and *Pythium ultimum* Trow, none of which appeared to be the primary cause of the injury. However, the soft decay which followed the dry rot no doubt increased the speed of the bulb's decline.—NEIL ALLAN MacLEAN, State College of Washington, Pullman, Washington.

The Occurrence of South American Leaf Blight of Hevea Rubber Trees in Mexico.—Hevea rubber tree plantings in Central America north of Costa Rica and in Mexico have been considered as being free from infection by *Dothidella ulei* P. Henn., the cause of South American leaf blight of Hevea.[1] Although many plantings in these countries have been under close observation since 1941, leaf blight has not been found in any of them until recently.

On September 26, 1946, the presence of South American leaf blight was discovered by the writer and T. D. Mallery in a seedling nursery near Teapa, Tabasco, in southern Mexico. Indications were that the infection was not more than 3 months old. In an attempt to trace the origin of the disease in Mexico, a survey was started of all the Hevea plantings in the Tabasco-Chiapas rubber zone. New infections were found in most of the one-year-old seedling nurseries near Teapa. Since these nurseries had originated from disease-free seed of mature Hevea trees at El Palmar in the state of Veracruz, it was assumed that the infection had originated from some of the early Hevea plantings in Tabasco or Chiapas. A few scattered old trees near Teapa had only relatively new infections.

In the states of Tabasco and Chiapas there are 10 or more isolated plantings of Hevea rubber trees which were started about 1910 and later abandoned. Although only a few trees have survived in most of these plantings, the one at Las Palmas, in the northwestern part of the state of Chiapas, contains 18,000 trees. An inspection of the trees at Las Palmas revealed evidence of leaf-blight infection which was at least two years old. The trees had been inspected and found to be blight-free in 1941. This evidence indicated that some other planting in the zone was the original source of infection, since there had not been any known introductions of foreign Hevea material into the zone during recent years.

Inspections of the other old Hevea plantings known to exist in the Tabasco-Chiapas zone revealed the probable source of South American leaf blight of *Hevea* in Mexico. A planting of approximately 800 trees located along the Chumulá River near Chuctiepá in northeastern Chiapas, approxi-

[1] Langford, M. H. South American leaf blight of Hevea rubbertrees. U. S. Dept. Agr. Tech. Bul. 882. 1945.

mately 40 miles east to northeast of Yajalon, Chiapas, was heavily infected with leaf blight and evidence indicated that it had been infected many years. This planting had been made about 1910 with small stumped seedlings, the origin of which has not been determined. It is probable that the fungus was introduced with this original planting material. There are several possibilities as to the mode of spread of the pathogen to the other Hevea plantings in the Tabasco-Chiapas zone. The probable spread was from numerous volunteer seedlings, growing underneath the old Hevea trees at Chumulá, which were obtained by a farmer near Teapa and sold to other farmers in the region.

On October 11, 1946, initial infections of leaf blight were first observed by Julius Matz in the large seedling nurseries at the Campo Experimental de Hule at El Palmar in the state of Veracruz. Evidence points to the exchange of workers as the probable means by which the pathogen was introduced into the El Palmar plantings from the Tabasco-Chiapas zone.

A close survey of the Hevea plantings in Guatemala during November, 1946, failed to show any leaf blight in those plantings. The plantings in Honduras, El Salvador, Nicaragua, Haiti, and the Dominican Republic apparently are also blight-free.

The hitherto restricted occurrence of leaf blight in Mexico greatly simplified the initiation of the Hevea planting program in that country since 1941. Its present widespread distribution, while somewhat unexpected, should not interfere with the progress of the program, since there is a good supply of resistant Hevea clones available in Mexico for use in top budding the high-yielding but susceptible clones being planted.—W. J. MARTIN, formerly in Division of Rubber Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Urocystis agropyri on Phleum pratense.[1]—Stripe smut of timothy in the northeastern United States is commonly caused by *Ustilago striiformis* (West.) Niessl. Formation of linear sori that eventually rupture and lead to shredding of leaves is a characteristic symptom of the disease. In the fall of 1944, Kreitlow and Cassell[2] collected a smut on timothy near Brandon, Vermont, that was indistinguishable macroscopically from *U. striiformis*, but when spores of the smut were examined with a microscope they were found to consist of spore balls resembling those of *Urocystis agropyri* (Preuss) Schroet. No previous report of this fungus on *Phleum pratense* L. was found.

The identical symptoms of the two diseases suggested that perhaps some specimens of timothy identified macroscopically as infected by *Ustilago*

[2] Kreitlow, K. W. and Robert C. Cassell. *Phleum pratense* L., a new natural host for *Urocystis agropyri*. U. S. Dept. Agr. Pl. Dis. Reptr. **29**: 365. 1945.

striiformis might in reality be attacked by *Urocystis agropyri.* Travel restrictions during the war prevented further collections, but in June, 1946, and in 1947, smutted timothy was collected in several northeastern states. The field where *Urocystis* was first obtained was revisited in 1946, but the timothy was gone and no smutted plants were located. Of the specimens collected in 1946, all but one were identified as *U. striiformis.* The exception was a collection of *Urocystis agropyri* obtained near Walpole, New Hampshire. No specimens of *Urocystis* were obtained among the samples of smutted timothy collected in 1947.

A highway distance of approximately 75 miles separated the two samples of *Urocystis agropyri* collected on timothy. In this connection, a collection of smutted timothy made by Dr. Charles Drechsler in 1917 near Warrensburg, Missouri, should be mentioned. The causal organism was identified as *Urocystis agropyri* and a specimen was deposited in the Mycological Collections of the Division of Mycology and Disease Survey. No report of the collection was published. Drechsler's specimen was examined and the spores found comparable to those obtained in Vermont and New Hampshire. This suggests the smut is not localized or restricted to the northeast.

To date, 26 samples of smutted timothy have been collected in the following states: Maine, New Hampshire, Vermont, New York, Connecticut, Massachusetts, New Jersey, West Virginia, and Pennyslvania. Microscopic examination, however, showed that all but two of the specimens were infected by *Ustilago striiformis.*

Spore balls of the smut on timothy resembled *Urocystis agropyri,* from other species of grasses, in general characteristics such as size, shape, and color. There was, however, a predominence of single-spored spore balls.[3] The limited herbarium material available prevented extensive experimental work with the smut. However, tests were conducted to induce germination of spores by exposing them to a temperature of 30° C. in a moist chamber for 10 to 14 days or by soaking spore balls in different dilutions of benzaldehyde. None of these measures yielded germinating spores.

The few specimens of *Urocystis* collected on timothy to date suggest that this smut occurs infrequently on timothy. However, unless microscopic examinations are made, the similarity in symptoms of the two smuts may result in attributing some cases of infection by *Urocystis agropyri* to *Ustilago striiformis.*—K. W. KREITLOW, U. S. Regional Pasture Research Laboratory, State College, Pennsylvania.

[3] In a personal communication, Dr. George W. Fischer verified the identification of the smut and pointed out the predominence of single-spored spore balls.

THE PREVENTION OF BLACK STEM RUST OF WHEAT

A. F. El-Helaly

INTRODUCTION

Wheat is the most important winter crop in Egypt. It is grown all over the country from the Mediterranean to the borders of the Sudan, and it is used entirely for local food. In 1937 there were about 860,000 feddans[1] in "the Delta" of Lower Egypt, 330,000 feddans in Middle Egypt, and 350,000 feddans in Upper Egypt (1). The chief varieties are Hindi, which are hard wheats of increasing importance, and Baladi, which are soft wheats. Sowing takes place in October and November, and harvest occurs in April and May.

Black stem rust caused by *Puccinia graminis* Pers. is the most serious disease of wheat in Egypt. It severely attacks the Hindi varieties of wheat, while the Baladi wheats are comparatively resistant or immune. It is so destructive to Hindi wheats that during epidemics in certain localities the entire crop may be lost.

Destructive epidemics of black stem rust occur during certain seasons, while other seasons are comparatively free from the disease. It was thought at one time (6) that black stem rust appeared every other year, alternating with yellow rust caused by *Puccinia glumarum* (Schm.) Erikss. and Henn., but this was found to be not the case. In most years, however, black stem rust is a limiting factor in wheat production.

Black stem rust appears, generally, in the warmer winters between February and March and may then affect yield very severely. The attack varies greatly, in intensity, from field to field.

It is believed, because the aecial stage of the disease has not yet been found in Egypt, that infection occurs each year by urediospores either hibernating, *i.e.*, overwintering, in the soil or being blown across the Mediterranean from Europe. Jones (29) by exposing, periodically, slides smeared with vaseline or glycerine on aeroplanes flying between Cairo and Alexandria at about 1000 ft. altitude, stated that the largest numbers of stem rust spores were found on slides exposed in March, about a fortnight before the first reported attack on wheat in Egypt.

OBJECT OF INVESTIGATION

The production of rust-resistant varieties, which is the ideal method of controlling rust diseases, requires several years of continuous work to produce a variety which combines resistance to rust with such desirable characters as good quality, good yield, etc. Therefore, it would be of great economic importance if direct methods of control could be devised.

[1] The feddan is equivalent to 1.47 acres.

The principal object of the present work is to investigate the possibility of preventing black stem rust of wheat by one of the common methods of plant protection.

METHOD AND MATERIAL

Various fungicides were dusted and sprayed on the plants in the field just before or at the time the first signs of the disease appeared on the crop. Dusts were applied by means of small hand dusters.   Spraying was carried out by means of "Holder" sprayers or by means of "Bean Junior" spraying pumps.   The former sprayers were used in small plot experiments, the latter in large plot experiments.

Latin square and randomized block experiments surrounded by wide belts of the same crop were used, and the analysis of variance was employed in analyzing the yield data of most of the experiments and in determining the significance of the results obtained.

Weight and volume of one thousand kernels taken at random to represent each treatment were also recorded in many cases.

The net profit or loss per feddan in Egyptian pounds[2] for each treatment was recorded in most cases.   This was calculated taking into consideration that the feddan was given, on the average, 1500 liters of the diluted fungicide for each application.   The cost of labor and of tear and wear of the sprayer for the application, in normal peace time, is about £0.25 and the price of the ardeb[3] of wheat grain is usually about £1.50.

Hindi D wheat variety, which is susceptible to stem rust, was used in most cases.   It is mentioned when other wheat varieties were used.   The seed was obtained from the Propagation Section of the Ministry of Agriculture.   The following commercial and home-made fungicides were used:

1. Flowers of sulphur.
2. Kolodust: a proprietary colloidal sulphur dust.
3. Wettable sulphur: a proprietary sulphur spray.
4. Kolofog: a proprietary colloidal sulphur which contains 30 per cent sulphur (6 lb. Kolofog + 8 lb. chemically hydrated lime + 100 gallons of water).
5. Sulsol: a proprietary colloidal sulphur compound which contains 40 per cent sulphur by weight.
6. Amberine: a proprietary solution of polysulphidal sulphur.
7. Lime-sulphur (home-made):   ·
     Diluted: 1 kilo quick lime + 2 kilos sulphur + 12.5 liters water.
     Concentrated: 1 kilo quick lime + 2 kilos sulphur + 6.25 liters water.
8. Bouisol: a proprietary colloidal copper compound which contains 15 per cent copper by weight.
9. Bordeaux mixture in powder form, mixture C.
10. Bordeaux mixture (home-made): 1 per cent = 4 lb. copper sulphate + 4 lb. quick lime + 40 gal. water.

[2] The Egyptian pound is valued at $4.94.
[3] The ardeb is equivalent to 5.619 bushels (U. S.).

The work to be described in this paper was carried out during the wheat growing seasons from 1935 to 1942 in the Field Stations of the Ministry of Agriculture at El-Serw, Sakha, Gemmeza, Dokki, Giza, Seds, Mallawi, Shandaweel, and Mataana, and in the Estate of H.H. Prince M. A. Haleem at Fariskour.

OCCURRENCE OF THE DISEASE IN EGYPT

The first record of the disease in Egypt, in 1920, was reported by Briton-Jones (6, 7). He attributed the disease to *Puccinia graminis* Pers. Fahmy (See 39) reported *Puccinia graminis* Pers. on wheat in 1923. Melchers (39) found it on wheat at Giza in 1928. Jones (29) reported *Puccinia graminis* Pers. on wheat in 1935 and stated that "it is the most serious disease of wheat in Egypt."

DISTRIBUTION OF THE DISEASE

Black stem rust of wheat is usually very severe in Lower Egypt where loss in yield may sometimes amount to 50–100 per cent. It decreases gradually southwards becoming rare in Upper Egypt.

TABLE 1.—*The percentage of black stem rust infection in wheat sown on three different dates in various localities in Egypt*

Localities	Rust infection in wheat sown on		
	Oct. 15	Nov. 1	Nov. 15
	Per cent	*Per cent*	*Per cent*
Lower Egypt			
El-Serw	60	80	80
Sakha	60	70	75
Gemmeza	35	45	50
Middle Egypt			
Dokki	20	25	25
Seds	6	10	10
Mallawi	2	3	3
Upper Egypt			
Shandaweel	Tr.	Tr.	Tr.
Mataana	Tr.	Tr.	Tr.

This is illustrated in table 1 which contains data obtained from similarly treated experiments made in different localities all over the country. These experiments were actually designed to find out the effect of date of sowing on the amount of disease. Data were taken from experimental plots sown on October 15, November 1, and November 15. These dates of sowing were chosen because they fall within the period during which normal sowing takes place in Egypt.

The disease was, for the first time, very severe in the Luxor region of Upper Egypt in 1941. Study of the conditions prevailing in this region at that time and examination of the crop there showed that the disease was severe on plants which had received much water or which were growing near irrigating canals.

EFFECT OF CULTURAL CONDITIONS ON AMOUNT OF DISEASE

Observations in the field and statements of farmers indicate that the amount of the disease is influenced by the cultural conditions prevailing during the growth of the wheat plants. Factors which arise in this connection are date of sowing, number of waterings, and manurial treatment.

Date of Sowing

It has been noticed during the earlier investigations of the disease that date of sowing plays an important part in the incidence of the disease.

TABLE 2.—*The black stem rust infection and yield of wheat sown on six different dates during 1939, 1940, and 1941 at Dokki, in Middle Egypt*

Date of sowing	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	Ardeb	Per cent	Grams	Cc.
1939					
Oct. 9	40	9.06	187.58	36.38	27.0
Oct. 15	50	9.02	186.75	36.75	27.4
Nov. 1	70	5.79	119.88	26.85	20.3
Nov. 15	85	3.73	77.23	18.45	14.8
Dec. 1	80	1.00	20.70	8.81	8.3
Dec. 15	65	0.34	7.04	4.10	4.7
Mean		4.83	100.00		
S.E.		0.307	6.36		
Sig. diff.		0.97	20.10		
1940					
Oct. 1	20	9.01	139.19	36.20	27.5
Oct. 15	25	8.95	138.11	36.12	27.3
Nov. 1	35	8.42	129.94	34.61	26.3
Nov. 15	40	7.65	118.05	30.31	23.3
Dec. 1	50	3.79	58.49	18.39	14.5
Dec. 15	50	1.05	16.20	11.77	9.5
Mean		6.48	100.00		
S.E.		0.458	7.068		
Sig. diff.		1.35	20.83		
1941					
Oct. 1	18	5.00	90.04	34.74	26.0
Oct. 15	37	7.87	142.03	33.10	24.9
Nov. 1	50	8.08	146.00	26.36	20.9
Nov. 15	60	7.31	132.01	22.90	18.7
Dec. 1	53	3.47	62.07	14.84	13.1
Dec. 15	35	1.46	26.04	9.61	9.3
Mean		5.53	100.00		
S.E.		0.375	6.78 ·		
Sig. diff.		1.11	20.00		

The plants of the late sowings usually have heavier attacks and give much lower yields.

An illustration of the effect of date of sowing on amount of disease and consequently on yield was clearly seen in an experiment which was designed for this purpose in 1939–1940 at Dokki. Successive sowings were made Oct. 9 and 15, Nov. 1 and 15, Dec. 1 and 15, 1939. Plots were 3 × 7 meters, and each treatment was repeated six times. The data obtained are in table 2 and illustrated in figure 1.
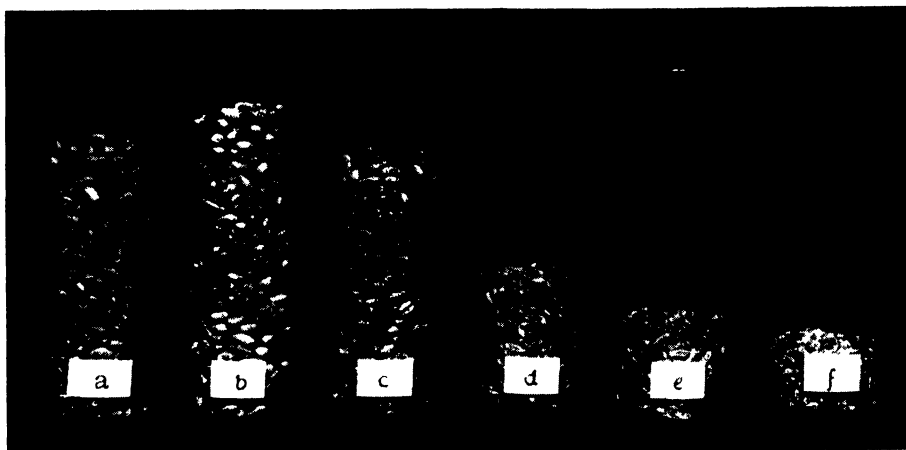
FIG. 1. Six samples, each containing 1250 wheat kernels, collected at random from crops sown on different dates in 1939—a, Oct. 9; b, Oct. 15; c, Nov. 1; d, Nov. 15; e, Dec. 1; and f, Dec. 15.

The same experiment was repeated in different localities in 1940–1941 and in 1941–1942. Table 2 also contains data obtained from the two experiments at Dokki in 1940–1941 and 1941–1942, respectively.

The weight of the yield tends to diminish with successive sowings. A careful study of the data presented in table 2 will show that, though the effect of date of sowing on yield is pronounced, the differences in yield are not always well correlated with the amount of disease. It seems that the plants of the early sowings escaped most of the ill effects of the disease. The disease appeared on the plants of the early sowings after the formation of the seed, i.e., when the crop was about to ripen. This left a short time for the disease to parasitize the host and, thus, though rust was severe, it had little effect on the yield.

To find out this, the plants of the experiment of 1941–1942 at Dokki were observed closely from the time of sowing to the time of harvest. Records of the dates of the first appearance of the disease on the plants and the first appearance of the ears are shown in table 3.

TABLE 3.—*The time elapsing between heading and the appearance of black stem rust infection in wheat sown on different dates in 1941 at Dokki, in Middle Egypt*

Date of sowing, 1941	Date of the appearance of		Period between appearance of ears and rust
	Ears	Rust infection	
			Days
Oct. 1	Dec. 20	Feb. 17	60
Oct. 15	Jan. 6	Feb. 19	45
Nov. 1	Feb. 4	Feb. 23	20
Nov. 15	Feb. 17	Feb. 26	10
Dec. 1	Mar. 3	Mar. 3	0
Dec. 15	Mar. 13	Mar. 13	0

It appears from the records given in table 3 that the period between the appearance of the ears and the initial attack of the disease on the same crop diminishes gradually with the successive sowings. It was 60 days in the first sowing, 45 in the second, 20 in the third, and 10 in the fourth. The disease and the ears appeared at the same time on the plants of the last two sowings.

The ears, or the kernels, of the plants of the first sowings had, therefore, much more time to grow during the absence of the disease. The kernels were nearly mature at the time of the appearance of the disease on the crop. They almost attained, therefore, their full size, so that a normal yield was produced.

In the last sowings, the disease had, from the beginning, a very big share of the food provided by the plants for the formation of the kernels. The kernels, thus, matured abnormally and became shrivelled, and there was an enormous decrease in the yield.

TABLE 4.—*The effect of irrigations on development of black stem rust and on yield of wheat at Dokki, Egypt, in two growing seasons*

Growing season and number of waterings	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	Ardeb	Per cent	Grams	Cc.
1939–1940					
One, at sowing time	30	4.18	70.73	25.36	19.3
Two	50	6.59	111.51	27.25	20.8
Three	55	6.67	112.86	28.01	21.0
Four	65	6.22	105.25	26.56	20.3
Five	65	5.85	98.98	26.33	20.0
Mean		5.91	100.00		
S.E.		0.586	9.92		
Sig. diff.		1.91	32.33		
1940–1941					
One, at sowing time	30	4.71	77.09	30.12	23.2
Two	60	5.64	92.32	31.41	24.0
Three	70	6.61	108.18	33.64	26.0
Four	70	6.65	108.84	34.57	26.5
Five	70	6.94	113.58	34.75	26.5
Mean		6.11	100.00		
S.E.		0.408	6.678		
Sig. diff.		1.26	8.41		

Number of Waterings

It has been mentioned that the outbreak of the disease in the Luxor region in 1941 was much more severe in the crops which had received more water during their growth. It was also observed by Pantanelli (43) that infection was very severe in cases where much water was absorbed by the plant, in proportion to the weight of its absorptive system, during the period immediately preceding inoculation, and that an increased concentration of the nutrient solution diminished susceptibility in so far as it reduced the absorptive activity of the roots.

To find out whether the number of waterings given to the plants during

their growing season affects in any way the development of stem rust disease, two similar experiments were designed at Dokki, one in 1939–1940 and the other in 1940–1941, on 3×7 meter plots. Each treatment was repeated five times. The results are in table 4.

The amount of the disease increased with the increase in the number of waterings given to the crop during its growing period. There was no appreciable difference in the yields for the different treatments. The expected increase in yield of the crop receiving one watering, where rust was light, was not attained. This was due to shortage in the soil moisture resulting from the single watering received by the plants. The loss in yield due to the insufficient single watering was equal to, if not more than, that caused by rust in other treatments. In the first case yield was affected by lack of water, while in the other cases the plants were weakened by rust. In both cases the grain shriveled as a result of too rapid ripening of the crop.

The number of waterings seemed to have also an indirect effect on the spread of the disease. The irrigated soil usually produced a humid atmosphere around the plants themselves. This promoted germination of the spores and thus facilitated secondary infection.

Manurial Treatment

It is a general experience of farmers that nitrogenous manures increase the incidence of the disease. The value of manuring as a preventive of rust was demonstrated by many workers on plant diseases. Hicke (26) reported that wheat plants receiving potassium salt together with ammonium sulphate remained almost entirely free from infection, while those receiving only ammonium sulphate were heavily infected. Phosphoric acid and lime also tended to counteract the development of yellow rust, which had been observed to occur with particular severity on wheat following clover and other Leguminosae. Günther (23) stated that infection with yellow rust was most severe in fields where wheat followed clover and root crops, because of the excess of nitrogen and the depletion of potash and phosphoric acid in the soil. Fertilization with ammonium compounds reduced the incidence of infection but induced lodging. The greatest resistance was in crops growing on soil to which potash and phosphoric acid had been applied. Mass (36) reported that potash increased resistance to yellow rust. Finger (15) stated that although late applications of nitrogen were usually considered to increase susceptibility, yet plentiful and uniform applications greatly reduced the incidence of infection but also resulted in lodging and delayed maturity. Liberal applications of potash strengthened the plants and increased their resistance to yellow rust. Remy and von Meer (45) concluded that potassium chloride gave better control of yellow rust than potassium sulphate. There appears to be a correlation between the high chloride content of the salt (acting, perhaps, by acidification of the cell sap) and the resistance to yellow rust. Schilcher (47) found that potash and phosphorus fertilizers reduce the incidence of rust which was promoted, on the other hand, by nitrogenous fertilizers.

In order to find out the effect of manurial treatments on stem rust of wheat, an experiment was carried out at Dokki during 1939–1940, using 3 × 7 meter plots which had been differently manured. The treatments, for

TABLE 5.—*The black stem rust infection and yield of wheat given various manurial treatments at Dokki, Egypt, in 1939–1940*

Treatment and time applied	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
Calcium nitrate					
After sowing	50	4.85	90.99	25.52	19.5
Before first watering	50	6.23	116.89	29.21	22.5
One-half after sowing, one-half before first watering	60	6.94	130.21	30.18	22.8
One-half after sowing, one-half before second watering	60	5.99	112.38	28.39	21.3
One-half before first watering, one-half before second watering	60	5.71	107.13	28.16	21.1
One-third after sowing, one-third before first watering, and one-third before second watering	62	6.03	113.13	28.43	21.3
Potassium sulphate					
After sowing	50	4.85	90.99	25.52	19.5
Before first watering	60	4.80	90.06	25.45	19.4
One-half after sowing, one-half before first watering	65	5.44	102.06	27.10	20.5
One-half after sowing, one-half before second watering	55	4.81	90.24	25.45	19.5
One-half before first watering, one-half before second watering	55	4.55	85.37	25.31	19.4
One-third after sowing, one-third before first watering, and one-third before second watering	60	5.07	95.12	26.50	20.5
Superphosphate					
Before sowing	50	4.63	86.87	25.51	19.8
Farmyard manure					
Before sowing	50	5.34	100.19	26.91	20.8
Control	50	4.80	90.06	25.45	19.3
Mean	...	5.33	100.00	.	.
S.E.	.	0.465	8.72	.	.
Sig. diff.	...	1.35	25.32	.	.

triplicate plots, were as follows: Calcium nitrate, 200 kilos per feddan; Potassium sulphate, 200 kilos per feddan; Superphosphate, 200 kilos per feddan; Farmyard (Baladi) manure, 15 cubic meters per feddan; Control, nothing added.

The data (Table 5) show that there is a particular advantage in using the nitrogenous manure. The plants manured with calcium nitrate, except those of the first treatment, gave slightly better yield. There was not much difference in the amount of the disease in all treatments.

Two experiments using only the nitrogenous manure (Calcium nitrate) were designed at Dokki during 1940–1941 and 1941–1942 in order to ascertain the value of the nitrogenous manure on the incidence of black stem rust. The data obtained are in tables 6 and 7. The expected increase in yield in the manured plots was not attained because the amount of the disease on the manured plants was more than the amount on the controls.

TABLE 6.—*The black stem rust infection and yield of wheat given calcium nitrate at various times and grown at Dokki, Egypt, in 1940–1941*

Time at which calcium nitrate (200 k. per feddan) was applied	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	Ardeb	Per cent	Grams	Cc.
After sowing	68	2.79	105.28	13.59	11.9
Before first watering	65	2.39	90.18	12.90	11.2
Before second watering	65	2.88	108.67	13.70	11.9
One-half after sowing and one-half before first watering	70	2.76	104.15	13.51	11.8
One-half after sowing and one-half before second watering	70	2.46	92.83	13.12	11.4
One-half before first watering and one-half before second watering	75	2.45	92.45	12.99	11.4
One-third after sowing, one-third before first watering, and one-third before second watering	75	2.68	101.15	13.32	11.5
Control	40	2.75	103.73	13.39	11.6
Mean		2.65	100.00		
S.E.		0.268	10.113		
Sig. diff.		0.79	29.81		

VARIETAL RESISTANCE

The Hindi varieties of wheat are susceptible to stem rust disease, while the Baladi varieties are comparatively resistant or even immune.

In an experiment at Dokki during 1941–1942 a number of commercial varieties were tested with reference to this point. Observations also were taken from variety experiments made in the same year all over the country. The data obtained are in table 8.

Among the varieties tested under the different conditions of these experiments the least susceptible to black stem rust disease were the Baladi varieties. The Hindi varieties were much more susceptible to the disease.

USE OF FUNGICIDES FOR DISEASE CONTROL

Much work has been done in an attempt to prevent attacks of rust diseases by chemical means. Kellerman (30) was among the earlier workers

on this subject. He treated wheat, oats, and barley with flowers of sulphur, potassium sulphide, iron chloride, and Bordeaux mixture, and got negative results. Many other investigators, including Pammel (42), Hitchcock and Carleton (27, 28), Bolley (5), Cobb (9), Galloway (16), Pearson (44), and Noiret (41), followed his footsteps using different fungicides to find out whether or not the development of rust could be prevented, but they did not get definite results.

TABLE 7.—*The black stem rust infection and yield in wheat supplied with varying amounts of calcium nitrate and grown at Dokki, Egypt, in 1941–1942*

Calcium nitrate added to the feddan at the rate of	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
100 kilos: before first watering	21	10.88	111.00	35.37	27.4
100 kilos: one-half before first watering and one-half before second watering	21	11.14	113.06	34.57	26.8
200 kilos: before first watering	24	9.05	92.03	33.54	26.3
200 kilos: one-half before first watering and one-half before second watering	24	9.18	93.67	33.97	26.5
300 kilos: before first watering	24	9.96	101.06	32.34	26.2
300 kilos: one-half before first watering and one-half before second watering	26	9.63	98.02	33.33	25.6
300 kilos: one-third before first watering, one-third before second watering, and one-third before third watering	26	9.06	92.04	32.78	25.5
400 kilos: one-fourth before first watering, one-fourth before second watering, one-fourth before third watering, and one-fourth before fourth watering	31	9.24	94.02	32.10	25.1
Control	18	10.10	103.00	36.13	27.9
Mean		9.80	100.00		
S.E.		0.798	8.14		
Sig. diff.		2.28	23.02		

Eriksson and Henning (14) gave a detailed summary of the earlier investigations on the possibility of controlling rusts by the use of chemicals, and they concluded that, although partial control of rust is possible by the use of fungicides, practical control is not feasible.

The results of the experiments of Kightlinger (31) demonstrated clearly the possibility of controlling cereal rusts by dusting growing plants with sulphur. He pointed out that spraying is impracticable. Lambert and

Stakman (34), and Gassner and Straib (17) also recognized the impracticability of spraying for the control of rust diseases of small grains.

Bailey and Greaney (2) succeeded in controlling leaf and stem rust of wheat by dusting the growing crop with sulphur. The works of Kightlinger and Whetzel (32) and of Lambert and Stakman (33) confirmed the possibility of preventing rust by dusting with sulphur. Deutelmoser (10) recommended spraying with Bordeaux mixture for the prevention of rust of peas, beans, celery, and asparagus. Bailey and Greaney (3, 4), Sibilia (48), Güssow (24), Mencacci (40), Roussakoff (46), Lambert and Stakman (34), Lehman and Poole (35), Greaney (18, 19), Mains (38), Stakman and Person (50), and Broadfoot (8) found that rusts of wheat and oats were considerably reduced by dusting the plants in the field with sulphur (Mencacci used

TABLE 8.—*The black stem rust infection in several varieties of wheat grown at five different places in Egypt in 1941–1942*

Variety	Origin of variety	Percentage rust infection at				
		Dokki	Mazghouna (Giza)	Gemmeza	Sakha	El-Serw
Baladi 116	Single plant selection	Tr.	Tr.	Tr.	Tr.	Tr.
Baladi Bouhi	Do	8	.		...	.
Hindi Maarad	Do	25	.	.	. ..	.
Hindi D	Do	35	25	60	55	80
Hindi 62 (Dahabi)	Do	20	30	65	60	70
Kazouria	Foreign variety	15	.	.		...
Giza 121 (Mabrouk)	Giza 7 × Baladi 42	25	. 8	40	35	45
Giza 129	Do × Hindi 39		3	50	70	20
Giza 130	Do × Hindi 39		3	10	50	10
Giza 131	Do × Hindi 39		3	5	50	5
Giza 133	Do × Hindi 12		2	15	25	15
Hindi Abiad (Tosson)	Single plant selection	25	. ..	.	.	. .

cupric sulphur), which was applied by means of hand, horse-drawn, and aeroplane dusters.

Schilcher (47) found that while Bordeaux mixture proved ineffective in combating the brown rust of wheat, some benefit was derived from unoiled calcium cyanamide dust, though accompanied by burning of the leaves. Grooshevoy and Maklakova (22) stated that dusting with sulphur can be financially sound only in regions where rusts are particularly severe and in years of heavy epidemics. The work of Greaney (20 and 21), Mackie (37), and Sibilia (49) confirmed the conclusion that sulphur dust is effective in the prevention of wheat rusts. El-Helaly (12 and 13), in testing the value of different fungicides for bean rust control, found that spraying with one-half per cent Bordeaux mixture could be considered as the most efficient and profitable method for controlling rust and chocolate spot (11) of beans in Egypt. Hart and Allison (25) used borax (sodium borate), picric acid, para-toluenesulphonylamide, and ortho-toluenesulphonylamide

for stem rust control and found that the two last named gave the most promising results. The data obtained are considered to justify further experimentation with the two toluene compounds. Straib (51) concluded that it was as yet impossible, on the basis of his greenhouse trials, to forecast the commercial possibilities of spraying with picric acid, ortho- and para-toluolsulphonamide, acridin, and 0.2 per cent borax.

Preliminary Experiments.—A preliminary experiment was designed at Giza using a susceptible wheat variety, Hindi 12, which was sown as a protective belt for a checker experiment. The plants were already slightly infected at the beginning of the treatments which commenced on March 12, 1936. Sprays were applied at weekly intervals, while dusting with sulphur was performed twice a week. Each treatment was repeated twice. Treated and nontreated plots were alternately arranged. The results taken at harvest time, May 11, 1936, are given in table 9.

TABLE 9.—*The black stem rust infection in wheat grown in duplicate plots at Giza, Egypt in 1935–1936 and treated with various fungicides*

Fungicides	Percentage rust infection	
	Plot 1	Plot 2
Nontreated	81	97
0.4 per cent Sulsol + 0.4 per cent soap	28	4
Nontreated	32	76
0.4 per cent Bouisol	5	7
Nontreated	81	88
Sulphur, 15 kilos per application per feddan	63	45
Nontreated	97	77
5 per cent diluted lime-sulphur	11	5
Nontreated	93	90
1 per cent Bordeaux mixture, mixture C	8	9
Nontreated	81	90

The data show that the effect of sprays in preventing stem rust is pronounced: the incidence of the disease was reduced about 80 per cent. On the other hand, sulphur dust had only slight effect on the disease.

This work was continued during 1936–1937 and 1937–1938 to verify the results in hand. Weight of yield was taken into consideration.

The experiments which were designed for this purpose at Gemmeza and Fariskour in 1936–1937 and at Dokki in 1937–1938 gave similar good results concerning the control of the disease. The disease was slight in these two seasons and appeared, also, late on the plants, particularly in 1937–1938.

Tables 10 and 11 present the data obtained from the Gemmeza and Dokki experiments of 1936–1937 and 1937–1938, respectively. Plots 3 × 4 meters were used, and each treatment was repeated three times.

The effect of the disease on the crop was slight because it appeared late on the plants of the two experiments. Nevertheless, the results obtained give a hopeful indication of the possibility of preventing black stem rust with chemicals. The following observations are worth mentioning:

1. Dusting with flowers of sulphur and with Kolodust had a very slight effect on controlling stem rust.

TABLE 10.—*The black stem rust infection and yield in wheat sprayed with fungicides. Experiments at Gemmeza, Egypt in 1936–1937*

Fungicides and concentrations	Interval between applications	Treatment commenced on					
		February 3, 1937			March 5, 1937		
		Total no. of applications	Rust infections	Av. yield per feddan	Total no. of applications	Rust infections	Av. yield per feddan
	Weeks		*Per cent*	*Ardeb*		*Per cent*	*Ardeb*
Amberine .	2	6			4		
0.2 per cent[a] .			55	8.2		55	7.4
0.1 per cent[a]			55	8.5		65	7.6
0.05 per cent[a] .			70	8.3		70	6.3
Dilute lime sulphur	2	6			4		
5.0 per cent[a] .			15	9.6		15	9.6
2.5 per cent[a]			20	8.6		20	9.0
1.25 per cent[a] .			25	8.6		40	8.6
Sulsol	2	6			4		
0.3 per cent[a]			30	8.1		50	7.8
0.15 per cent[a]			45	8.9		60	7.6
0.075 per cent[a] .. .			48	8.7		70	8.3
Bouisol	2	6			4		
0.2 per cent			65	7.1		45	8.0
0.1 per cent			60	7.1		60	7.3
0.05 per cent			55	8.6		65	7.2
Bordeaux mixture C	2	6			4		
1.0 per cent			20	9.4		50	8.8
0.5 per cent			25	8.3		60	8.6
0.25 per cent			40	8.7		60	8.3
Amberine .	4	3			2		
0.2 per cent[a] .			55	8.3		65	7.7
0.1 per cent[a]			70	7.5		75	7.8
0.05 per cent[a] .			70	7.1		75	7.7
Dilute lime sulphur	4	3			2		
5.0 per cent[a] .			25	8.9		40	8.2
2.5 per cent[a]			40	8.7		50	7.9
1.25 per cent[a]			45	8.7		55	7.7
Sulsol .	4	3			2		
0.3 per cent[a]			45	7.2		65	6.5
0.15 per cent[a]			40	8.1		65	7.1
0.075 per cent[a]			50	7.8		70	8.1
Bouisol	4	3			2		
0.2 per cent			50	8.3		65	6.8
0.1 per cent			60	8.3		70	7.0
0.05 per cent			60	7.9		70	6.7
Bordeaux mixture C	4	3			2		
1.0 per cent			40	8.8		60	8.0
0.5 per cent			45	8.2		70	7.2
0.25 per cent			60	8.7		70	7.3
Control (Not treated) ...			70	7.2		70	7.2

[a] A 0.2 per cent soap was added to the fungicide.

2. A few sprays gave rather satisfactory control of the disease. Lime-sulphur seemed to be the most effective in preventing stem rust.

TABLE 11.— *The black stem rust infection and yield of wheat dusted or sprayed with sulphur compounds. Experiments at Dokki, Egypt in 1938–1939*

Treatment, sulphur compound, and amount used on a feddan	Number of applications and dates, 1939	Rust infection	Av. yield per feddan
		Per cent	Ardeb
Dust			
Flowers of sulphur	Three: Mar. 28,		
20 lb.	Apr. 6 and 28	40	9.57
40 lb.		40	9.49
80 lb.		35	9.81
Control		40	9.22
Kolodust	Three: Mar. 28,		
20 lb.	Apr. 6 and 28	40	9.73
40 lb.		40	9.71
80 lb.		35	9.97
Control		40	9.65
Spray			
Kolofog	One: Mar. 27		
20 lb.		40	9.25
40 lb.		35	10.19
80 lb.		35	10.15
Control		40	9.74
Kolofog	Two: Mar. 27		
20 lb.	and Apr. 5	35	10.23
40 lb.		35	10.10
80 lb.		35	10.30
Control		40	9.87
Kolofog	Three: Mar. 27,		
20 lb.	Apr. 5 and 28	45	8.74
40 lb.		35	10.20
80 lb.		30	10.74
Control		35	10.10
Wettable sulphur	One: Mar. 27		
20 lb.		35	10.92
40 lb.		40	9.48
80 lb.		40	10.03
Control		40	9.99
Wettable sulphur	Two: Mar. 27		
20 lb.	and Apr. 5	35	10.24
40 lb.		40	9.44
80 lb.		30	10.83
Control		40	9.74
Wettable sulphur	Three: Mar. 27,		
20 lb.	Apr. 5 and 28	25	11.92
40 lb.		25	11.05
80 lb.		25	11.55
Control		35	10.39
Dilute lime-sulphur	One: Mar. 27		
5 per cent		10	10.95
2.5 per cent		20	10.11
1.25 per cent		25	10.79
Control		40	9.93
Dilute lime-sulphur	Two: Mar. 27		
5 per cent	and Apr. 5	5	11.57
2.5 per cent		10	10.52
1.25 per cent		20	10.73
Control		45	9.36
Dilute lime-sulphur	Three: Mar. 27,		
5 per cent	Apr. 5 and 28	Tr.	11.96
2.5 per cent		5	11.66
1.25 per cent		15	10.91
Control		45	9.37

3. Although the difference in amount of yield between the treated and the nontreated plots was small, yet it was sufficient for us to conclude that these sprays are somewhat effective in preventing the disease. The difference was more pronounced with lime-sulphur.

This work was continued in 1939–1940 in order to study the relative fungicidal value of the available sulphur dusts and sprays and to determine which of them was the most suitable for preventing the disease. Different doses of dusts and dilutions of sprays as well as different numbers of applications were tried.

TABLE 12.—*The black stem rust infection and yield of wheat treated with flowers of sulphur or with Kolodust at weekly or fortnightly intervals. Experiments at Dokki in 1939–1940*

Sulphur compound and amount used	Total no. of applications and time between applications	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
		Per cent	Ardeb	Per cent	Grams	Cc.
Flowers of sulphur	Six: 1 week					
20 lb.		60	5.78	96.81	26.33	19.8
40 lb.		55	5.34	89.44	26.79	20.4
80 lb.		60	5.83	97.65	25.36	19.3
Control		60	5.97	100.00	26.33	20.0
Flowers of sulphur	Three: 2 weeks					
20 lb.		65	6.23	104.35	25.25	19.3
40 lb.		55	5.54	92.79	26.14	19.8
80 lb.		60	6.34	106.19	25.47	19.3
Control		55	6.38	106.86	25.73	19.6
Kolodust	Six: 1 week					
20 lb.		55	6.03	101.00	25.29	19.3
40 lb.		55	6.19	103.68	29.98	20.4
80 lb.		55	7.15	119.76	27.13	20.5
Control		55	5.35	89.61	25.51	19.4
Kolodust	Three: 2 weeks					
20 lb.		60	5.83	97.65	25.91	19.8
40 lb.		65	5.70	95.47	26.88	20.4
80 lb.		60	6.23	104.35	26.98	20.5
Control		60	5.67	94.97	25.84	19.8
Mean			5.97	100.00	......	....
S.E.			0.498	8.34	. .	... .
Sig. diff.		..	1.438	24.09	.. .	. ...

Dust Fungicides.—An experiment, using 3×7 meter plots, was carried out at Dokki in 1939–1940 to ascertain the value of flowers of sulphur and Kolodust on the prevention of black stem rust disease and, eventually, on the yield. Dusts were applied at the rate of 20, 40, and 80 lb. dust per feddan at weekly and at fortnightly intervals, each treatment being repeated three times. Table 12 contains the data obtained.

The results confirmed the conclusion already mentioned. Dusting the plants with either flowers of sulphur or Kolodust had slight effect on the disease and, consequently, on the yield.

Spray Fungicides.—The results of the preliminary experiments gave

TABLE 13.—*The black stem rust infection and yield of wheat sprayed with fungicides, and the net profit or loss resulting from treatment. Experiments at Dokki in 1939–1940*

Spray material, concentration or amount used on a feddan	No. of applications	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels	Net profit or loss
		Per cent	Ardeb	Per cent	Grams	Cc.	£
Dilute lime-sulphur							
5 per cent	1	75	5.54	111.23	19.10	15.0	+ 2.600
	2	15	8.47	172.50	27.27	21.0	+ 6.235
	3	5	10.55	214.86	30.88	23.3	+ 9.925
2.5 per cent	1	80	4.45	90.63	18.76	14.8	+ 0.935
	2	45	6.91	140.73	25.56	19.6	+ 4.135
	3	15	8.38	170.67	28.69	21.7	+ 5.520
1.25 per cent	1	85	4.18	85.31	16.91	13.6	+ 0.740
	2	60	6.45	131.36	21.30	16.8	+ 3.765
	3	40	6.74	137.27	24.79	19.3	+ 4.740
Control	1	95	3.40	69.24	14.28	11.9	0.000
	2	100	3.50	71.28	16.06	13.8	0.000
	3	100	2.72	55.39	13.13	10.9	0.000
Kolofog							
80 lb.	1	95	4.38	89.20	15.68	12.7	+ 0.085
	2	80	4.40	89.61	23.22	18.5	− 0.640
	3	85	4.92	100.20	18.34	15.2	− 2.490
40 lb.	1	95	4.16	84.72	13.77	11.3	− 0.175
	2	85	4.76	94.90	18.54	14.8	+ 0.300
	3	85	4.56	92.87	17.08	14.2	− 0.050
20 lb.	1	95	3.93	80.04	15.01	12.5	− 0.440
	2	90	4.76	96.94	17.43	13.8	+ 0.500
	3	85	5.16	105.09	17.79	14.3	− 0.450
Control	1	95	3.99	81.26	13.41	11.3	0.000
	2	95	3.96	80.65	13.63	11.5	0.000
	3	90	4.76	96.94	16.77	13.7	0.000
Wettable sulphur							
80 lb.	1	75	4.61	93.89	14.42	12.2	+ 0.915
	2	85	5.28	107.55	19.62	15.2	+ 0.405
	3	80	5.04	102.64	19.44	15.9	− 1.230
40 lb.	1	90	4.03	82.07	15.93	13.0	− 0.020
	2	90	5.19	105.70	17.36	14.0	+ 1.070
	3	80	5.67	115.47	19.98	15.6	+ 0.645
20 lb.	1	90	4.04	82.28	17.24	13.8	+ 0.660
	2	95	5.19	105.70	17.13	14.2	+ 1.470
	3	80	5.48	111.60	19.73	15.5	+ 0.225
Control	1	100	3.30	67.20	13.41	11.3	0.000
	2	100	3.61	73.52	13.76	11.9	0.000
	3	95	3.94	80.24	14.01	12.0	0.000
Bordeaux mixture (home-made)							
1 per cent .. .	1	95	3.64	74.13	16.36	13.3	− 0.865
	2	80	5.57	113,44	18.71	14.9	+ 0.505
	3	70	5.87	119.55	20.55	16.5	+ 1.620
0.5 per cent	1	90	3.89	79.22	18.64	13.2	− 0.370
	2	80	4.25	86.55	18.45	14.8	− 1.235
	3	80	4.48	91.24	16.34	13.0	− 0.105
0.25 per cent	1	95	4.29	87.37	17.20	14.0	+ 0.240
	2	90	4.20	85.53	16.88	13.7	− 0.131
	3	85	4.40	89.61	13.67	11.2	− 0.040
Control	1	95	3.89	79.22	14.02	11.4	0.000
	2	90	4.58	93.27	15.47	12.7	0.000
	3	95	3.81	77.59	16.14	13.2	0.000
Mean	......	.... .	4.91	100.00	....	. .....	. ..
S.E. . .:.	......	. ...	0.610	12.42	...	.. .	. ..
Sig. diff.	......	.	1.73	25.22			

great promise to the possibility of preventing black stem rust disease by spraying the plants in the field with fungicides. This line of investigation was continued during the wheat growing season of 1939–1940, in order to determine the cheapest, most effective and economic spray for stem rust control. Table 13 gives the result of an experiment carried out at Dokki, using 3 × 7 meter plots. Different numbers of sprays with different concentrations in each case were tried. The treated plots received either one
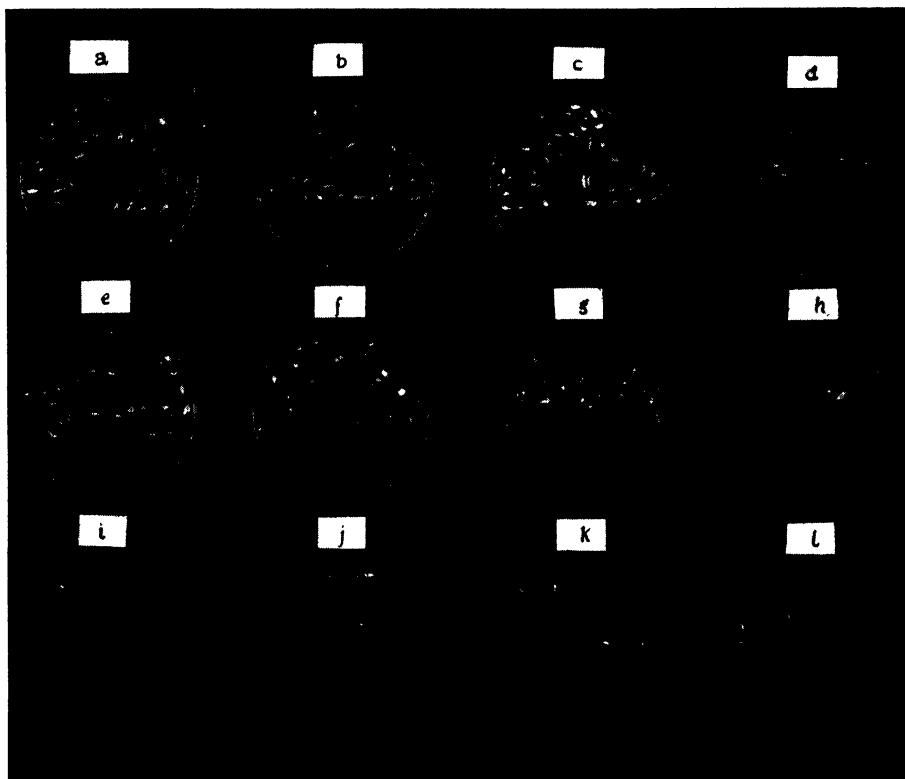
FIG. 2. Twelve samples, each containing 75 wheat kernels, collected at random from crops receiving lime-sulphur sprays in 1940. Samples a, b, and c were from crops receiving 3 sprays (March 12 and 26, and April 10) of 5 per cent, 2.5 per cent, and 1.25 per cent, respectively, of diluted lime-sulphur. Lot d was from the control plot. Samples e, f, and g were from plots receiving 2 sprays (March 12 and 26) of 5 per cent, 2.5 per cent, and 1.25 per cent, respectively, of diluted lime-sulphur. Lot h was from the control plot. Samples i, j, and k were from plots receiving a single spray (March 12) of 5 per cent, 2.5 per cent, and 1.25 per cent, respectively, of diluted lime-sulphur. Lot 1 was from the control plot.

spray on March 12, 1940, or two sprays on March 12 and 26, 1940, or three sprays on March 12, March 26, and April 10, 1940. Each treatment was repeated three times.

An examination of the results given in table 13 leads to the following conclusions:

1. Kolofog and Bordeaux mixture had a very slight effect on the disease

and consequently on the yield (Fig. 4, A and 3, B). There was a financial loss in most cases.

2. The return profit derived from the use of wettable sulphur fluctuated. The cost of treatment was high and, therefore, did not justify the small profit gained by the increase in yield (Fig. 4, B).

3. Excellent results were obtained with lime-sulphur. Yield increased
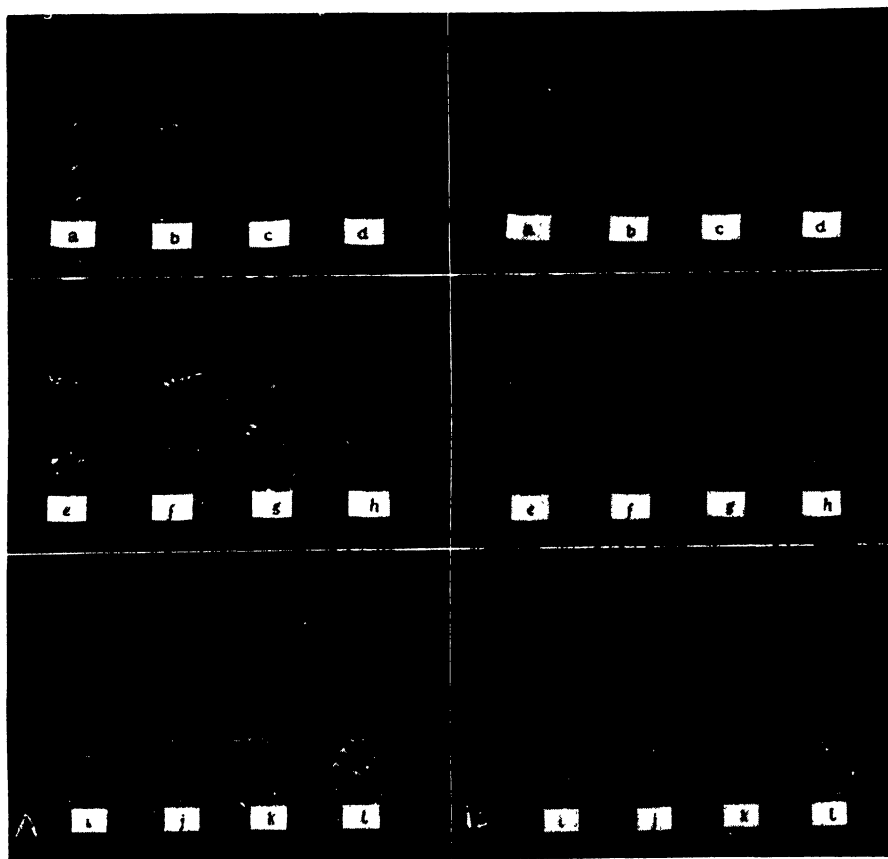
FIG. 3. Samples of 1500 wheat kernels collected at random from plots receiving dilute lime-sulphur sprays (A) or Bordeaux mixture sprays (B) to prevent stem rust.

In A, lots a, e, and i received 5 per cent lime-sulphur, lots b, f, and j received 2.5 per cent, lots c, g, and k received 1.25 per cent, and lots d, h, and l were controls.

In B, lots a, e, and i received 1 per cent Bordeaux mixture, lots b, f, and j received 0.5 per cent, lots c, g, and k received 0.25 per cent, and lots d, h, and l were controls.

In both A and B, lots a, b, and c received 3 sprays, on March 12 and 26 and on April 10, 1940. Lots e, f, and g received 2 sprays, on March 12 and 26. Lots i, j, and k received single sprays, on March 12.

to a marked degree (Fig. 2 and 3, A), and a high net profit was obtained in all cases.

4. Spraying with lime-sulphur proved to be the most satisfactory method for controlling black stem rust of wheat. It resulted in the biggest yield and the highest net profit. Yield differed according to con-

centration of spray, number of applications, and amount of disease. The net profit varied accordingly from about one to ten Egyptian pounds per feddan.
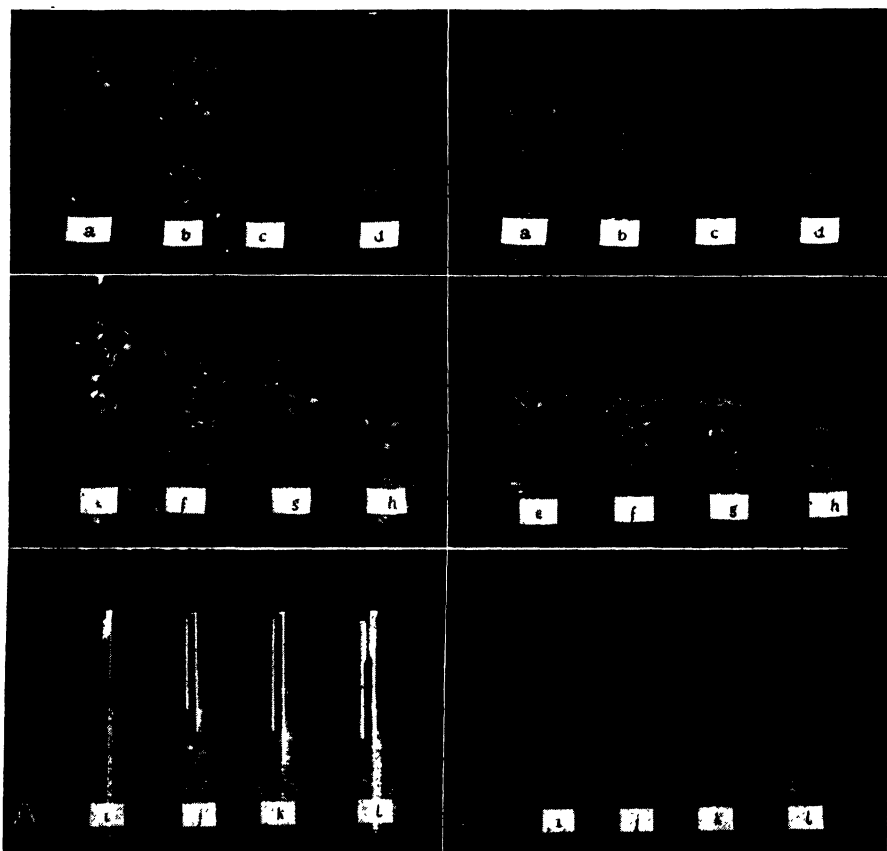
FIG. 4. Samples of 1500 wheat kernels collected at random from plots receiving Kolofog sprays (A) or wettable sulphur sprays (B) to prevent stem rust.

Lots a, e, and i received 80 lb. of the fungicide per feddan; lots b, f, and j received 40 lb.; lots c, g, and k received 20 lb.; and lots d, h, and l were controls.

Lots a, b, and c received 3 sprays, on March 12 and 26 and on April 10, 1940. Lots e, f, and g received 2 sprays, on March 12 and 26. Lots i, j, and k received single sprays, on March 12.

LIME-SULPHUR FOR THE PREVENTION OF THE DISEASE

In the experiments already described, the amount of disease was successfully reduced when plants were sprayed with 5 per cent, 2.5 per cent and 1.25 per cent diluted lime-sulphur. There was a big increase in the yield and a high net profit in all cases.

It was, therefore, advisable to study the effect of lime-sulphur on the disease with a view to determining the best concentration, the requisite number of sprays, the suitable interval between applications, and, finally, the time when the treatment should begin.

Two similar experiments, using 3×7 meter plots, were designed in 1940–1941, and 1941–1942 at Dokki. Each treatment was repeated six times. Tables 14 and 15 present the results obtained. The data given are highly

TABLE 14.—*The black stem rust infection and yield of wheat sprayed with concentrated lime-sulphur, and the net profit or loss resulting from such treatment. Experiments at Dokki in 1940–1941*

Strength of concentrated lime-sulphur	Date of application 1941	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels	Net profit or loss
		Per cent	Ardeb	Per cent	Grams	Cc.	£.
2.5 per cent	March 5	35	9.78	115.73	31.16	23.5	+ 3.890
1.25 per cent		50	8.94	105.79	29.08	22.5	+ 2.750
0.625 per cent		55	8.62	102.01	28.91	22.4	+ 2.330
Control		80	6.78	80.23	26.77	21.0	0.000
2.5 per cent	March 25	50	8.67	102.60	29.31	22.6	+ 2.300
1.25 per cent		60	8.15	96.44	29.35	22.7	+ 1.640
0.625 per cent		60	8.12	96.09	27.45	21.2	+ 1.655
Control		80	6.73	72.54	25.55	19.8	0.000
2.5 per cent	April 12	60	8.75	98.81	28.39	21.3	+ 1.610
1.25 per cent		70	7.70	91.12	28.36	21.3	+ 0.155
0.625 per cent		75	7.31	86.50	28.29	21.3	+ 0.155
Control		75	7.27	86.03	27.22	21.0	0.000
2.5 per cent	March 5	20	10.08	118.13	32.29	25.0	+ 2.360
1.25 per cent	and 25	30	9.53	112.78	31.39	24.0	+ 1.735
0.625 per cent		45	8.93	105.68	30.55	23.5	+ 0.955
Control		75	7.72	91.36	26.94	21.0	0.000
2.5 per cent	March 5	40	8.90	105.32	29.63	23.0	+ 1.930
1.25 per cent	and	45	8.68	102.72	29.94	23.2	+ 1.910
0.625 per cent	April 12	50	8.56	101.30	29.26	22.8	+ 1.780
Control		80	6.80	80.45	25.58	19.8	0.000
2.5 per cent	March 25	40	9.95	117.51	32.12	25.1	+ 2.425
1.25 per cent	and	45	9.42	110.29	30.71	23.7	+ 1.870
0.625 per cent	April 12	50	8.81	104.26	30.43	23.5	+ 1.075
Control		75	7.52	88.99	27.11	21.2	0.000
2.5 per cent	March 5 and	5	10.26	121.42	33.13	26.0	+ 3.900
1.25 per cent	25, and	15	10.09	119.40	32.92	25.6	+ 4.005
0.625 per cent	April 12	40	8.56	101.30	29.59	22.8	+ 1.890
Control		80	6.44	76.21	26.51	20.8	0.000
Mean		....	8.45	100.00	....	.. ..	.. ..
S.E.		.	0.494	5.846	... ..	.. ..	.. ..
Sig. diff.		.	1.38	16.33		.	.

significant and the following conclusions may be deduced:

1. Satisfactory reduction of stem rust of wheat was attained with most treatments.

2. The net profit per feddan due to the reduction of the disease varies from £1.00 to £4.00 according to the degree of the incidence of the disease, concentration of spray, number of sprays, date of initial spray, and interval between applications.

3. Spraying was successful when applied at the right time, just before or at the time of the appearance of the disease in the field (during the first half of March).

4. One spray is sometimes profitable; two and three sprays with two or three weeks interval are efficient in the reduction of the disease.

5. The most effective control and the highest net profit were attained by the use of the higher concentrations.

6. The data (Table 15) confirmed the general conclusions already put forward. This is in spite of the fact that the disease was not so severe, thus, having little effect on the untreated plants which produced, accordingly, good yield. The difference in yield per feddan between the treated and the untreated crops was, therefore, not great.

7. The net profits derived from the application of the 2.5 per cent concentrated lime-sulphur and, to a less extent, the 1.25 per cent justify their use for the prevention of the disease even in years when the disease is slight.

TABLE 15.—*The black stem rust infection and yield of wheat sprayed with concentrated lime-sulphur, and the net profit or loss resulting from such treatment. Experiments at Dokki in 1941–1942*

Strength of concentrated lime-sulphur	Date of application 1942	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels	Net profit or loss
		Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*	*£.*
2.5 per cent	March 9	15	12.96	96.17	35.84	26.9	− 0.610
1.25 per cent		20	13.83	105.49	35.97	26.8	+ 0.725
0.625 per cent		25	13.31	·101.52	35.27	26.5	+ 0.005
Control		30	13.02	99.38	34.60	26.0	0.000
2.5 per cent	March 25	10	13.89	105.94	36.23	27.1	+ 0.800
1.25 per cent		15	12.93	98.62	36.20	27.0	− 0.490
0.625 per cent		20	12.72	97.00	36.87	27.1	− 0.430
Control		30	12.95	98.07	34.26	25.9	0.000
2.5 per cent	April 12	20	13.22	100.08	34.85	26.0	− 0.610
1.25 per cent	·	20	12.74	97.01	34.15	26.2	− 0.490
0.625 per cent		25	12.18	92.09	33.95	25.6	− 0.430
Control		25	13.46	102.06	34.57	25.9	0.000
2.5 per cent	March 9	2	14.15	107.09	35.04	26.3	+ 2.285
1.25 per cent	and 25	5	13.72	104.06	36.77	27.3	+ 1.870
0.625 per cent		15	12.59	96.00	35.75	27.1	+ 0.290
Control		25	11.82	90.16	33.33	25.0	0.000
2.5 per cent	March 9	5	13.57	103.04	35.72	27.4	+ 0.630
1.25 per cent	and	10	13.14	100.02	36.17	27.3	+ 0.220
0.625 per cent	April 12	15	12.91	98.04	34.69	26.4	− 0.005
Control		· 25	12.34	94.01	33.86	25.6	0.000
2.5 per cent	March 25	5	13.53	103.02	35.17	25.5	− 0.130
1.25 per cent	and	15	13.38	102.00	34.27	26.1	− 0.125
0.625 per cent	April 12	20	13.04	99.04	34.27	26.4	− 0.515
Control		30	12.81	97.07	35.92	26.7	0.000
2.5 per cent	March 9 and	Tr.	14.28	108.09	37.31	27.8	+ 1.170
1.25 per cent	25, and	5	13.21	100.07	37.11	27.6	− 0.075
0.625 per cent	April 12	10	13.00	99.01	35.75	26.5	− 0.190
Control		25	12.28	93.06	34.78	25.9	0.000
Mean		......	13.11	100.00	....	....	....
S.E.		......	0.299	2.20	....	....	....
Sig. diff.		..... ·	0.84	6.04	....	....	....

DISCUSSION AND SUMMARY

Black stem rust of wheat is widely distributed in Lower and, to a less extent, in Middle Egypt, where it sometimes causes considerable damage and serious losses to the crop.

Certain cultural conditions influence the amount of the disease. The disease increases with the increase in number of waterings given to the plants, and, also, with the increase in the nitrogenous manure added to the soil. The plants of the early sowings usually escape the effect of the disease, because it appears on them after the formation of the seed, *i.e.*, when the crop is about to ripen.

The Hindi varieties of wheat are susceptible to black stem rust disease; while the Baladi varieties are comparatively resistant or even immune.

In testing the efficiency and the economic value of the fungicide for black stem rust prevention, it was found that dusting the growing plants in the field with flowers of sulphur and Kolodust and spraying them with Kolofog, wettable sulphur, Amberine, Sulsol, Bouisol, and Bordeaux mixture had slight effect on the disease and, consequently, on the yield. Spraying with lime-sulphur, on the other hand, was the most satisfactory method for reducing the amount of stem rust of wheat. It resulted in the biggest yield and the highest net profit. These values differ according to concentration of spray, number of applications, amount of disease and, finally, date of initial application.

The most effective control and the highest net profit were attained with the use of the higher concentrations of lime-sulphur. The increase in yield resulting from spraying with 2.5 and 1.25 per cent concentrated lime-sulphur covers the cost of tear and wear of the sprayer, the cost of material used and also labor, and finally leaves, in addition, some profit to justify its application. It is apparent that even in years when stem rust is slight (*e.g.*, 1941–1942) spraying with the two said concentrations will control rust satisfactorily and profitably. Spraying with each of the two said concentrations could therefore be considered as the most efficient and profitable method for controlling stem rust of wheat in small areas in Egypt.

Entirely satisfactory results were always obtained by applying the initial spray to the plants when rust was first detected in the district, and then by thoroughly protecting the plants until they were practically ripe.

Although one spray gave more or less satisfactory control of the disease, yet one or two other sprays with an interval of two or three weeks were profitable. Spraying should be started just before or at the time of the appearance of the first signs of the disease in the field. Too early and too late spraying had little effect on the prevention of the disease and, therefore, was of little economic value.

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THE ROLE OF PLANT RESIDUES IN THE ETIOLOGY
OF ROOT ROT[1]

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The incorporation of plant residues into the soil is known to affect, if not indeed to determine, the occurrence and severity of certain root rot diseases of plants. The types of organic matter present influence, in a manner not yet understood, the root rot of corn (17), the brown root rot of tobacco (1, 6), and the black root rot of strawberry (9).

The present investigation is based on the hypothesis that some root rots are initiated by the direct toxic action of plant residues; the hypothesis implies that the activities of soil organisms are secondary and are incident upon an initial injury which is of chemical origin.

Root rot, even so considered, remains a disease, not a symptom. Disease may be defined as an active, dynamic response by the plant to a set of abnormal environmental conditions; a nematode or microbial pathogen may or may not be a feature of this environment. A symptom is defined in static, rather than dynamic, terms; it is a visible, or at least measurable, abnormal state of the plant resulting from its prior response to the abnormal environment.

The germ theory of disease has without question led to results of the greatest theoretical and practical importance. Among the root rots there are indubitable cases of a one-to-one correspondence of disease and microbial pathogen, as in the take-all disease of wheat and the Phymatotrichum root rot of cotton. However, the root rots of strawberry and of pea are just as clearly not associated with the action of one or even a few microorganisms; the number of fungi described in the literature as causally related to these diseases is so large as to throw doubt on the whole method of approach.

Because of the common association of the word "rot" with the action of microorganisms, it might seem preferable to use a more neutral term in discussing root injury of chemical origin. It seems, however, that the same considerations apply here as in the case of the concept of disease. Most rots are caused by organisms, but exceptions occur, as in the case of autolysis after freezing. In the same way, the hypothesis of a chemical origin of certain root rots does not require a new term, only a realization that rots are not required by the very definition of the word to be caused by microorganisms.

The effect of plant residues on root rot may be, obviously, either to decrease or to increase disease incidence. With the former case we are not at present concerned, except to note that organic matter is known to decrease

the severity of Phymatotrichum root rot (12) and the take-all disease of wheat (7). In the latter case—increase in disease—there are at least four possible theories of the mode of action: (1) direct stimulation of the growth or activity of a pathogen; (2) changes in the microbial balance of the soil or the rhizosphere; (3) depletion or unavailability of soil nutrients during microbial decomposition of the added residue; and (4) the direct toxic action of the residue or its decomposition products. It is the last-named hypothesis that the experiments reported here are designed to test.

The early work of the Bureau of Soils on "soil toxins" (18) has been justly criticized (15) and is not of itself convincing. However, several recent reports (2, 3, 16) have demonstrated that certain constituents of normal plants may be toxic to the same or other plants.

Collison (4) and Collison and Conn (5) demonstrated that extracts of wheat straw were toxic to young seedlings in water culture, causing stunting and root discoloration. The experimental design eliminated nitrogen deficiency, acidity, and oxygen depletion from consideration as causes of the symptoms. The authors concluded that specific chemicals present in the straw were responsible for plant injury; composting of the straw resulted in their disappearance.

Extracts of partially decomposed timothy have been reported by Doran (6) to cause injury to tobacco roots; Gries (8) found that extracts of decomposing red clover were toxic to strawberry roots. In both these cases the toxicity was believed to result from microbial action on the plant residue involved. Newton and Young (14) reported preliminary data indicating that extracts of undecomposed alfalfa exert inhibitory effects on the growth of wheat in sand culture; Valleau et al. (19) found that the addition of corn roots to sand cultures of tobacco caused a root rot; Matthews et al. (13) were able to induce browning of tomato roots by the incorporation into the soil of timothy roots.

As a necessary preliminary to experimental test of the hypothesis that chemicals present in plant materials may cause root injury of the type under discussion, a bio-assay was devised which, it is believed, offers some promise as a tool in further research. Such an assay, it was realized, must be free of the complications introduced by microbial activity during the experiment and must in addition be independent of changes in available nutrients. If these two conditions are met, it is possible to eliminate from consideration the first three of the theories mentioned earlier, however applicable they may be in other cases.

The present paper describes the bio-assay method and the results of its application to study of the toxicity of certain plant residues before, during, and after decomposition.

MATERIALS AND METHODS

Plant residues studied included soybean (variety Manchu), sweet corn (variety Golden Cross Bantam), ladino clover, and perennial ryegrass.

Plants were harvested (tops only) in late fall, air-dried, ground coarsely, and stored in covered stone crocks.

Each plant residue was allowed to decompose at two moisture levels. The "dry" series was decomposed at a moisture level equivalent to 50 per cent of saturation; this value ranged from 63.2 per cent for clover to 80.2 per cent for corn. In this series the residues were incubated in crocks with loose covers. Moisture contents of all samples were determined weekly and adjusted when necessary to the original level. In the "wet" series dry plant material and tap water were added to Mason jars in the ratio 1:20; in this case the solids were completely submerged.

Decomposition proceeded without the addition of soil as inoculum; preliminary experiments showed that the addition of one per cent soil had no significant effect on the number and types of microorganisms present two weeks later. The temperature of incubation was 24°–27° C., with the exception of certain experiments carried out at 56° C. Samples for determination of pH, microbial populations, and toxicity were taken before the start of decomposition, 3 days later, at weekly intervals for six weeks, and at three-week intervals until the close of the experiment.

Bacteria, actinomycetes, and spore-forming bacteria were counted on dilution plates of Bacto nutrient agar after incubation for ten days at 24°–27° C. Yeasts and fungi were counted on acidified (pH 4.0) Bacto potato-dextrose agar after four days. The numbers of thermophilic bacteria and actinomycetes were determined by counting dilution plates of nutrient agar after two days at 56° C. The population of anaerobic bacteria was measured by the extension dilution method in a reducing agar medium.

The effects of plant residues on roots were assayed by a method developed for the purpose. Radish seed (variety Early Scarlet Globe) was germinated on moist filter paper. After 72 hours seedlings with radicles 2–3 cm. in length were placed in shell vials containing the extracts to be tested. After eight hours at room temperature the seedlings were removed for grading.

Radish seed offers several advantages for this assay. Germination is rapid; the abundance of root hairs makes for quick absorption of soluble materials; and the injury is of such a nature that accurate measure of it can be made rapidly. Of the other plants tested, both spinach and pea showed the same reaction to plant residues but were less easy to manipulate; onion seedling roots, on the other hand, failed to respond to plant tissue extracts.

Extracts were prepared by soaking the plant tissue to be tested in boiled and cooled tap water for 20 minutes; the solids were then filtered off. Two concentrations were prepared in every case: one gram (dry basis) per 20 ml. of water, and one gram per 40 ml. In all experiments the control consisted of roots of the respective plants soaked eight hours in boiled tap water.

The basis of the assay was the browning of the radish root caused by certain plant tissue extracts. For each of 20 roots an estimate was made

of the fraction of the root length which had been discolored by the extract. According to the grading system devised by Horsfall and Barratt (10), this proportion was expressed as a grade; the mean grade for each lot of 20 roots was then converted to "percentage browning" by reference to a prepared calibration curve.

Only brown discoloration was considered; the color ranged from light to dark brown. The intensity of color usually decreased as the fractional root length discolored became smaller, but no effort was made to separate treatments on the basis of color intensity. All plant extracts, whether causing browning or not, induced a water-soaking of the root tissue.

<div align="center">RESULTS</div>

Microbiological Population Changes

Counts of the common soil microorganisms were made at each sampling period. Results conformed in general with those of other workers (20) and need not be presented in detail. As will be brought out in the next section, toxicity of residues decreased with time, and this is believed to be associated with decomposition; but further correlation of injury with microbial populations was not evident. This is particularly striking in view of the very different floras occurring at the two different moisture levels and at the different times of sampling. In the "dry" series (50 per cent saturation), ammonification and the resultant high pH encouraged the development of bacteria and actinomycetes. In plant material submerged in water the growth of acid-forming bacteria was followed by profuse development of fungi; utilization of acid by the fungi resulted in a slow rise in pH and a gradual replacement of fungi by bacteria. The nature of the plant residue naturally affected the composition of the microflora, but again there was no apparent relation to root rot.

Apart from this negative finding, there is one point which is of interest with regard to the microbial flora of decomposing plant residues. West and Hildebrand (22) characterize the breakdown of soybean tissue as "fermentative" and that of red clover as "putrefactive," with, in the former case, a low pH and low bacterial population relative to the latter. These differences in type of breakdown were believed to have some relation to the amount of strawberry root rot in soils to which these residues were added.

The microbiological results in the present study do not bear out this contention; the pH was found to be more dependent on the moisture level than on the type of plant material. Small differences in pH did occur as a reflection of nitrogen content in cultures where ammonification was possible.

An experiment modelled after that of West and Hildebrand was designed further to explore the microbial factors involved in the decomposition of clover and soybean. Fresh soybean and red clover tissue, from plants grown 6 weeks in the greenhouse, was finely chopped and 2.5 gm. of

each (approximate 0.5 gm. dry matter) were placed in 50 ml. of tap water in a prescription bottle; soil (0.5 gm.) was added to each culture. After 14 days at 28°–30° C., pH and bacterial numbers were determined. The data, summarized in table 1, do not support the generalization that soybean tissue undergoes an "acid" or "fermentative" breakdown with low bacterial numbers. Under the conditions of this experiment soybean tissue after 14 days of decomposition was slightly more alkaline and supported a slightly larger bacterial population than red clover tissue.

Other experiments showed that the acid type of breakdown may be induced with either plant material if larger amounts of the dried residues (1.0–2.5 gm. per 50 ml.) are added to water in the same way. In all likelihood, the beneficial effect of soybean on strawberry root rot is to be ex-

TABLE 1.—*General characteristics of the decomposition of soybean and red clover tissue after 14 days at 28°–30° C.*

Plant[a]	pH		Bacteria per ml.[b] (Total No. × 10⁻⁶)
	Initial	Final	
Soybean	6.61	7.60	596.7
Red clover	6.68	7.25	80.7

[a] 2.5 gm. fresh tissue plus 0.5 gm. soil in 50 ml. tap water.
[b] Determined by dilution plating on Bacto nutrient agar.

plained in some other manner than the mechanism postulated by West and Hildebrand.

Toxicity of Plant Residues

Cold-water extracts of plant residues were tested for possible injurious effect on radish roots as described above. Data for several of the residues appear in figures 1 and 2.

Extracts of undecomposed ladino clover proved to be toxic, as shown by the high response at zero time (Fig. 1). Clover tissue allowed to decompose at 50 per cent of its moisture-holding capacity became slightly more toxic to roots during the early stages of decomposition; it is not certain whether fluctuations over short periods are significant, but the general trend of toxicity was upward for 5 weeks. From this time on, toxicity decreased rapidly, reaching a very low level at 9–12 weeks.

Extracts of clover decomposed at the high moisture level lost their toxicity to roots more rapidly than extracts from the drier series; the same fluctuations in toxicity early in the decomposition period were noted.

While, as pointed out previously, the pH data show that the two moisture levels governed two very different types of decomposition, in both cases the overall picture is one of destruction of the high initial toxicity, modified by microbial effects acting over short periods of time.

The other legume under test, soybean, had, in contrast to the clover, no detectable initial toxicity to radish roots. There was apparent in the early

stages of decomposition at both moisture levels a very slight toxicity presumably resulting from microbial activity. The maximum injury from a 1:20 extract did not, however, exceed 3 per cent, and the toxicity disap-
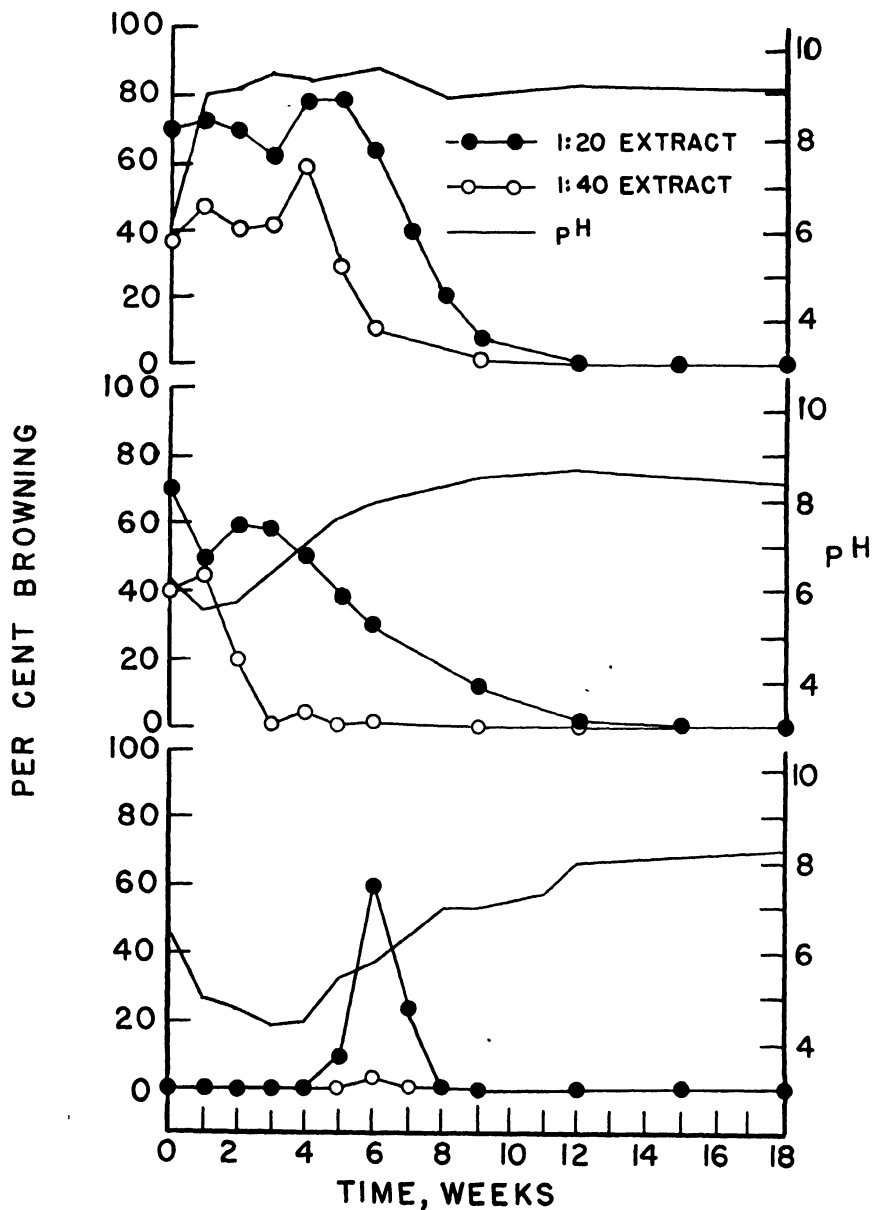
FIG. 1. Percentage browning of radish seedling roots in extracts of (1) clover tissue decomposing at 50 per cent saturation (top); (2) clover tissue decomposing under water (middle); (3) corn tissue decomposing under water (bottom).

peared in 4 weeks in one case ("dry" series), in 2 weeks in the other ("wet" series).

Extracts of undecomposed corn stover were not at all injurious to radish roots. Similarly, extracts from corn decomposed at 50 per cent of saturation were not toxic at any time over the 84-day period of observation.

In the corn stover decomposed under water, there was, as shown in figure 1, a period (28–42 days) during which extracts caused very noticeable injury to root tissue. This followed a period of minimal pH and the acidity may have been instrumental, directly or indirectly, in causing
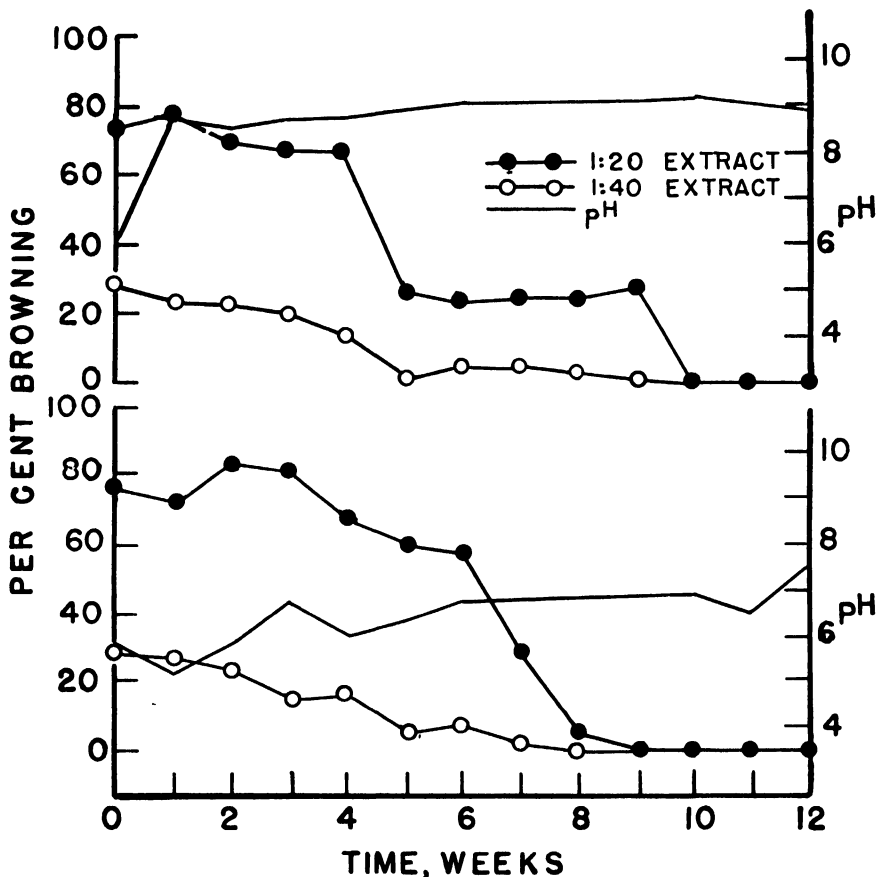
FIG. 2. Percentage browning of radish roots in extracts of ryegrass tissue decomposing at 50 per cent saturation (top), and in extracts of ryegrass tissue decomposing under water (bottom).

the injury. The effect is ascribed to microbial action, whether or not the acidity was a factor; at this stage in the decomposition fungi formed the predominant element in the microbial flora, and a steep decline in the number of fungi was coincident with the disappearance of injurious effects. It should be pointed out that the symptoms of injury to radish roots were not precisely similar to those caused by extracts of clover or ryegrass; the color of the affected tissue was more grayish, and there was a translucence not previously noted.

Extracts from undecomposed ryegrass were very injurious to radish roots, 80 per cent of the root length being damaged. As shown in figure 2, decomposition at both moisture levels resulted in progressive loss of toxicity, more rapid in the "wet" cultures than in the "dry." In the former case there was at 14 days a slight increase in injury from the 1:20 extract; since this increase did not occur with the 1:40 extract, it was probably of no significance. The course of pH change was similar to that in other plant materials and could not be correlated with changes in the toxicity of extracts.

The hypothesis that the disappearance of toxicity of clover and ryegrass residues is the result of microbial decomposition suggests that the process may be accelerated at high temperatures. Such conditions would

TABLE 2.—*The effect of decomposition at 56° C. on the toxicity of plant materials to radish roots*

Plant material[a]	Time, days	pH	Number of bacteria[b] (Total No. × 10⁻⁶)	Percentage browning of radish roots	
				1:20 Extract[c]	1:40 Extract[c]
Corn	0	6.52	< 0.001	0.0	0.0
	3	7.81	14.3	0.0	0.0
	7	8.00	2430.0	0.0	0.0
	14	7.68	4320.0	0.0	0.0
	21	8.39	2700.0	0.0	0.0
(Control)[d]	21	6.74	... ...	0.0	0.0
Ryegrass	0	5.78	0.001	71.0	22.5
	3	7.06	48.0	49.0	13.5
	7	7.49	894.0	6.3	0.0
	14	6.97	1170.0	3.1	0.0
	21	7.45	787.0	0.0	0.0
(Control)[d]	21	5.63	... ...	61.5	20.4

[a] Decomposed at a moisture content of 50 per cent of saturation.
[b] Determined by dilution plating on Bacto nutrient agar.
[c] Dry basis.
[d] Held at 56° C. for 21 days without addition of water.

approximate those of a compost pile during the period of active microbial thermogenesis. It is a commonplace of agronomic practice that composted organic matter is of greater benefit to crops than the same material before decomposition. Several factors probably contribute to the beneficial effect of composting, notably the preliminary breakdown of complex carbon compounds whose decomposition in the soil would result in a locking up of nitrogen. It seemed of interest, however, to determine the effect of compost-pile temperatures on the breakdown of the toxic factors under consideration in the present work.

The effect of decomposition at high temperature (56° C.) is shown in table 2. Samples of corn stover and of ryegrass were adjusted to a moisture level equivalent to 50 per cent of their water-holding capacity and incubated at 56° C. The moisture content was kept constant by daily deter-

minations of dry matter and addition of the required amount of water. At the high temperature dry matter losses were considerable, *e.g.*, about 40 per cent of the dry matter of ryegrass was lost in 14 days.

The toxicity of ryegrass to roots dropped very quickly as the result of high-temperature decomposition; at 21 days no injury could be noted with a 1:20 extract. At room temperature (Fig. 2), toxicity was still detectable after 84 days of decomposition.

The controls for the experiment on thermophilic decomposition were dry ground samples of the plant material incubated at 56° C. for 21 days. These controls were not, obviously, entirely satisfactory; conceivably the injurious material in ryegrass may be more sensitive to high temperature in the presence of water than in its absence. The similarity to decomposition

TABLE 3.—*The influence of the age of the plant on the toxicity of its water extract to radish seedling roots*

Plant	Date of collection	Age, days[a]	Percentage browning of radish roots[b]
Ladino clover	June 30	. ..	49.5
	July 15	..	56.0
	July 31	... .	25.0
	Aug. 15	..	49.5
	Aug. 31	....	73.2
Soybean	June 1	40	0.0
	June 15	55	0.0
	June 30	70	0.0
	July 15	85	0.0
	July 31	101	0.0
Corn	June 15	36	0.0
	July 15	66	0.0
	Aug. 15	97	0.0

[a] The ladino clover was planted the previous year.
[b] Radish seedlings immersed 8 hours in a 1:20 extract (dry basis) of the dried and ground tissue.

at room temperature is close, however, and it may be postulated that during the high-temperature decomposition the factor in ryegrass responsible for root injury is destroyed by microbial action.

In summary, extracts of ryegrass and of ladino clover were injurious to radish seedling roots; similar extracts of corn stover and of soybean tissue were not injurious. Decomposition resulted in a progressive decline in the toxicity of clover and ryegrass extracts; this decline was interrupted at times by secondary increases in toxicity caused apparently by microbial action, and was more rapid at 56° C. than at room temperature.

It will be noted that the two residues yielding toxic extracts were collected as green plants, the corn and soybean as dead plants. Waksman (21) found that young plants are higher in water-soluble constituents than are mature plants. The possibility exists therefore that the differences observed between plant materials are attributable to their age rather than to specific characters, since the test for toxicity depended on a water extraction.

The experiment reported in table 3 was designed to ascertain the influence of age of plant on the toxicity of water extracts to radish roots. The data show that neither young soybean nor young corn plants yield toxic water extracts. The toxicity of extracts from clover plants of different ages was variable but did not show any consistent effect of age of plant.

DISCUSSION

The laboratory assay used in the determination of toxicity of plant residue has obvious limitations. There are, however, two types of correlation with field experience which encourage belief in the validity of the assay.

First, the type of injury to radish seedling roots was very similar to that known in field root rots of other plants: watersoaking, browning, and death of tissues from the tip downward.

Second, and more important, there is a correlation with field experience in that soybean residues, known to reduce strawberry root rot (9) and corn root rot (17), were not injurious to radish. Conversely, clover residues, which generally increase root rot in soil (11, 17) proved to cause severe damage to radish root cells. The failure of corn residues to have a toxic effect may be a result of the fact that only stalks were used. Valleau et al. (19) found that previous corn crops increase tobacco root rot, and that dead corn roots are toxic to tobacco in sand culture.

The effect of extracts of plant residues on radish roots cannot be ascribed to nutrient deficiencies, to invasion of the roots by specific pathogens, or in all·likelihood to microbial activity in the extract itself. · The time period of 8 hours was too short to permit any of these factors to be operative. The injury therefore has the aspect of direct toxicity of a chemical or chemicals present in the cold-water extract of certain plant tissues.

It is important to note that the most injurious plant materials, clover and ryegrass, were so from the beginning. Microbial activity may have caused short-term increases in toxicity, but over the long run the decomposition of these materials resulted in concomitant destruction of the toxic factor. Decomposition by thermophilic organisms at 56° C. accelerated the destruction.

Undecomposed plant materials have occasionally been reported to cause injury to seedlings. Extracts of dried wheat straw and alfalfa hay were found to cause stunting of pea, corn, and barley seedlings (5); similar results have been obtained with alfalfa by other workers (14). These reports, together with the data here presented, suggest that, in studies of the effect of decomposing crop residues on plant growth, adequate controls must be included so that the effect of the plant material itself will not be interpreted as an effect of decomposition products.

A further artificial feature of the experiments here reported is the use of plant residues as such, when it is known that the concentration of such residues in soil is normally less than 10 per cent. To this objection it may

be argued that the extracts actually tested were dilute, and that in the soil local accumulations of undecomposed organic matter may occur in the vicinity of growing roots and serve to initiate root rot.

SUMMARY

Direct injury to root tissue by plant extracts can be assayed by exposing the radicles of 72-hour-old radish seedlings to the action of cold-water extracts of the ground plant tissue.

Extracts made from undecomposed ladino clover and from ryegrass caused browning of radish roots in 8 hours. Decomposition of these residues at two moisture levels resulted in a progressive disappearance of the factor causing the injury, although microbial action brought about temporary increases in toxicity. Decomposition of clover tissue at 56° C. accelerated the destruction of the toxic factor, presumably as a result of the activity of thermophilic bacteria. Apart from this case, studies of microbial populations in the decomposing residues failed to establish any significant association of particular groups of microorganisms with the appearance or disappearance of root injury.

Extracts from undecomposed corn stover did not cause root browning; microbial action under anaerobic conditions caused a transitory appearance of injurious activity.

Extracts of soybean tissue did not cause discoloration of radish roots, whether tested before, during, or after decomposition.

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EFFECT OF SEED TREATMENT ON THE GERMINATION
OF SOYBEANS

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The practice of treating soybean seed with fungicides to improve emergence, stand and yield is comparatively recent, and the results obtained from treated seed planted at different locations vary considerably. In general, however, seed treatments frequently have increased stand but rarely have increased yield.

In a recent paper, Hildebrand and Koch[2] reviewed the literature on the subject so well that a review need not be repeated here. They reported that with poor quality seed, treatment with Spergon increased emergence and yield. Under the conditions of their experiments, Spergon was consistently more effective than Arasan and Fermate in reducing disease or abnormality in early-season stands.[3]

As environmental factors influence results obtained in field experiments, it seemed worthwhile to conduct seed treatment tests in constant temperature rooms where certain environmental conditions or other variables could be controlled, and to compare results from these tests with results from certain plantings made in the field.

The experiments were conducted in two series, Series I with twelve seed lots of various varieties from different places in the Southern States and Illinois, and Series II with a single seed lot, Wood's Extra Early Yellow, from Virginia. In both of these series, portions of each seed lot were left untreated or were treated separately with Arasan (tetramethyl thiuram disulphide) or Spergon (tetrachloro parabenzoquinone) at the rate of two ounces per bushel, or with New Improved Ceresan (ethyl mercury phosphate) at the rate of one-half ounce per bushel. The seed in Series I was treated March 10, 1945, and two to three weeks later portions of each lot, treated and untreated, (designated Series I–A) were planted in constant temperature rooms held at 15°, 20°, 25°, and 30° C., respectively. Other portions of five of these twelve seed lots, treated and untreated, (designated Series I–B) were stored at room temperature in loosely covered glass jars and similarly planted in 1946, one year after treatment. In addition, after 15 months' storage, five similarly treated and untreated seed lots (designated Series I–C) were planted May 24, 1946 in the field at Beltsville, Maryland, 100 seeds per row, in 8-foot rows, replicated four times.

[2] Hildebrand, A. A., and L. W. Koch. Soybean diseases in Ontario and effectiveness of seed treatment. Phytopath. 37: 111–124. 1947.

[3] Hildebrand, A. A., and L. W. Koch. Seed treatment and other tests with soybeans in Ontario. (Abstr.) Phytopath. 36: 401. 1946.

The seed used in Series II was received in the spring of 1946. It had been grown in 1945 near Tidewater, Virginia and stored in an elevator. Seed of this lot was of poor quality and had been submitted to the writers because of the high percentage of discolored seeds.

The seed of Series II was separated into three classes according to the degree of discoloration: (1) beans with at least half of the area of the seed coat of each discolored; (2) those with less than half of the seed coat discolored; and, (3) those without discoloration. In classes (1) and (2), *Cercosporina kikuchii* Mats. and Tomo. was readily isolated from the beans with purple blotches, and oospores of *Peronospora manshurica* (Naoum.) Syd. formed conspicuous whitish crusts on many others. Portions of each of the three classes were left untreated or were treated and handled in the same manner as those in Series I, except that in Series II–A only one planting was made in the constant temperature rooms, and in Series II–B

TABLE 1.—*Influence of seed treatments at specific temperatures. The results with all seed lots were combined*

Treatment	Average number of seedlings emerged from 50 seeds in							
	Series I–A at[a]				Series I–B at[b]			
	15° C.	20° C.	25° C.	30° C.	15° C.	20° C.	25° C.	30° C.
Arasan	34.05**	37.00**	37.65**	36.87	6.87	19.87*	29.80**	28.40**
Spergon	30.54**	34.46**	36.26**	35.73	6.60	17.60	25.80**	27.47**
New Improved Ceresan	32.05**	36.94**	38.28**	36.92	13.93**	26.33**	31.60**	29.93**
No treatment	23.78	27.32	30.23	35.08	5.07	15.60	17.40	13.93

 * Significant at the 5 per cent level (1.98 required for I–A, 3.39 for I–B).
** Significant at the 1 per cent level (2.61 required for I–A, 4.48 for I–B).
[a] Planted 2 days to 3 weeks after treatment (12 seed lots).
[b] Planted 1 year after treatment (5 seed lots).

only 50 seeds were planted in the field in each 8-foot row, and there were only three replications.

In both Series I and Series II, the seed was planted in the constant temperature rooms in soil adjusted to 55 per cent of its water-holding capacity, and contained in small metal boxes as described by Leukel.[4] Fifty beans were planted 1½ inches deep in each metal box.

In both series, all emergence counts were made when about half of the seedlings were beginning to unfold their first trifoliolate leaves.

<div align="center">RESULTS</div>

The results obtained in Series I are given in tables 1, 2, and 3.

When the counts from the twelve seed lots used in Series I–A were combined, (Table 1) significant increases in stand, as compared with the untreated lots, were found from the three fungicides at 15°, 20° and 25° C., but not at 30° C. By combining the results at the four temperatures (Tables 2)

significant increases in stand were indicated from the three fungicides for most of the varieties. Exceptions were Chief (A) and Viking for Arasan; Patoka, Chief (C), and S 55–10 for Spergon; and Viking for New Improved Ceresan.

It appears from table 3 that seed treatment was more effective in increasing emergence from seed produced in the South than from that produced in Illinois. This contrast is particularly striking when the results from the variety Gibson and Nos. 42–91 and 42–53, all grown in the South, are compared with those from Chief (A), Patoka, and Viking, grown in Illinois. This is further substantiated by the fact that the seed of Chief (B), produced in Mississippi, were much lower in germination than were seed of

TABLE 2.—*Efficacy of treatments on different seed lots. Results at the four temperatures were combined*

	Average number of seedlings from 50 seeds planted							
	Series I–A 4 days after treatment with				Series I–B one year after treatment with			
Variety or seed lot	Arasan	Spergon	New Improved Ceresan	No treatment	Arasan	Spergon	New Improved Ceresan	No treatment
Chief (A)	37.85	38.65*	38.95*	35.08	——	——	——	——
Patoka	40.45**	38.33	40.40*	35.90	——	——	——	——
Viking	36.50	37.92*	36.35	33.83	——	——	——	——
Lincoln	47.50**	46.93**	48.28**	41.33	38.17**	34.34**	44.00**	27.67
Chief (B)	36.50*	37.58**	36.65*	32.83	——	——	——	——
Creole	46.50**	41.75**	46.40**	36.50	23.27**	24.08**	38.75**	13.00
Chief (C)	19.43**	14.75	21.43**	13.65	10.75	12.67*	9.67	8.50
S–100	39.30**	37.00**	39.75**	31.25	14.08**	10.67**	18.17**	5.49
S 55–10	25.10**	18.58	26.15**	15.68	——	——	——	——
Gibson	31.33**	24.83**	24.23**	15.68	——	——	——	——
42–91	36.25**	35.43**	35.08**	28.25	17.67**	15.33**	16.67**	10.09
42–53	40.00**	39.23**	38.90**	29.25	——	——	——	——

* Significant at the 5 per cent level (3.44 required for I–A, 3.79 for I–B).
** Significant at the 1 per cent level (4.52 required for I–A, 5.01 for I–B).

Chief (A) and (C), produced in Illinois, and that the greatest benefit from the seed treatment was obtained with the Mississippi-grown seed. It may be added that seed in the lot from Mississippi were more discolored and had less lustre than those in the lots from Illinois.

Emergence from seed of Mississippi-grown S 55–10 was significantly improved by treatment with Arasan at all four temperatures and with New Improved Ceresan at 20° and 25° C., but was not significantly improved by treatment with Spergon at any temperature. The differences at 25° C. are illustrated in figure 1. Apparently when seed germinate poorly or are heavily contaminated, Spergon is not so effective in improving emergence as are Arasan or New Improved Ceresan. This is apparent in seed lots of Creole and Chief (B), as well as in S 55–10.

From an analysis of variance, it was found that there was a significant difference in the reaction of the twelve seed lots (varieties) to temperature

and treatment, and that the interactions of variety × temperature, variety × treatment and temperature × treatment all had a significant F value.

The seed in Series I–A, which was planted soon after being treated, germinated better than that in Series I–B, which was planted a year after it was treated. The lower germination of the latter was especially pronounced for S 100 and 42–91 at 15° and 20° C. It is evident, however, as shown in table 3, that much better emergence was obtained from the treated stored seed than from the untreated stored seed, except for Chief (C) whose emergence was so poor that the counts were too small to show significant differences. On the whole, emergence from the treated seed exceeded that from the untreated, more in Series I–B than in Series I–A.

FIG. 1. Soybean seedlings of selection S 55–10 grown at 25° C. from 50 seeds treated with: A, Arasan; B, Spergon; C, New Improved Ceresan; and D, no treatment (control).

When the seedling counts from Series I–B (Table 1) were combined and subjected to the analysis of variance, significant improvement in emergence from each of the three treatments was evident at 25° and 30° C. At 20° C., significant increases in stand were obtained from treatments with Arasan and New Improved Ceresan, and at 15° C. from New Improved Ceresan only. New Improved Ceresan was the only fungicide to produce significant increases in emergence over the check at all temperatures. At 30° C., all fungicides increased stands significantly, which was not the case in Series I–A.

In Series I–B, there was a significant difference in the germination of varieties at different temperatures and treatments, and all of the interactions

TABLE 3.—Effect of variety, source of seed, seed treatment, soil temperature, and storage upon emergence in soybeans

Seed lot[a] and source	Treatment	Average number of seedlings emerged from 50 seeds in								Emergence in Series I-C[d] (Pct.)
		Series I-A at[b]				Series I-B at[c]				
		15° C.	20° C.	25° C.	30° C.	15° C.	20° C.	25° C.	30° C.	
Chief (A), from Edgewood, Ill.	Arasan	34.33	39.00	39.33	38.67	—	—	—	—	—
	Spergon	42.00**	35.67	38.33	38.67	—	—	—	—	—
	N. I. C.	34.33	39.33	41.33	40.67	—	—	—	—	—
	No treatment	30.33	34.67	36.67	38.67	—	—	—	—	—
Patoka, from Edgewood, Ill.	Arasan	42.00	38.33	40.67	40.67	—	—	—	—	—
	Spergon	39.00	38.33	37.00	39.00	—	—	—	—	—
	N. I. C.	40.33	40.67*	44.00*	36.67	—	—	—	—	—
	No treatment	37.00	33.00	36.67	36.67	—	—	—	—	—
Viking, from Edgewood, Ill.	Arasan	32.67	37.67	38.00	37.67	—	—	—	—	—
	Spergon	37.33**	39.00*	40.67	34.67	—	—	—	—	—
	N. I. C.	32.00	37.33	37.33	38.67	—	—	—	—	—
	No treatment	27.67	31.33	37.67	38.67	—	—	—	—	—
Lincoln, from Beltsville, Md.	Arasan	48.00*	49.33**	44.67	48.00	21.67	42.33*	45.00**	43.67**	89.75
	Spergon	45.67	47.67*	46.33*	48.00	16.67	37.00	41.00**	42.67**	87.50
	N. I. C.	49.67**	47.67*	46.67*	49.00	37.33**	46.67**	45.00**	47.00**	89.00
	No treatment	39.67	39.00	39.33	47.33	18.33	33.33	27.33	29.67	84.75
Creole, from Georgia	Arasan	45.67**	47.00**	45.00**	48.33	9.00	24.00*	32.67*	36.33**	88.25**
	Spergon	33.33	41.00	45.00**	47.67	8.67	19.67	33.33**	34.67**	88.25**
	N. I. C.	45.33**	47.33**	44.67**	48.33	23.00**	40.33**	45.33**	46.33**	92.00**
	No treatment	30.67	37.00	31.00	47.33	3.00	16.33	21.00	11.67	70.25
Chief (B), from Tunica, Miss.	Arasan	7.33	24.00**	25.67**	20.67	—	—	—	—	—
	Spergon	4.00	15.33	19.67	20.00	—	—	—	—	—
	N. I. C.	9.00	22.67**	26.33**	27.67	—	—	—	—	—
	No treatment	5.33	12.00	14.33	23.00	—	—	—	—	—
S 100, from Stoneville, Miss.	Arasan	39.33**	39.33*	40.33	38.33	0.33	10.00	27.00**	19.00**	64.25**
	Spergon	33.00**	37.33*	38.67	39.00	2.33	7.67	14.67	18.00**	66.25**
	N. I. C.	34.00**	41.33**	42.00*	41.67	3.00	17.00**	26.00**	26.67**	68.25**
	No treatment	23.67	30.33	34.33	36.67	2.00	5.33	13.33	1.33	48.75

TABLE 3—(Continued)

Seed lot[a] and source	Treatment	Average number of seedlings emerged from 50 seeds in								Emergence in Series I-C[d] (Pct.)
		Series I-A at[b]				Series I-B at[c]				
		15° C.	20° C.	25° C.	30° C.	15° C.	20° C.	25° C.	30° C.	
S 55–10, from Tunica, Miss.	Arasan	18.67**	22.67**	28.33*	30.67*	—	—	—	—	—
	Spergon	10.33	16.00	19.67	28.33	—	—	—	—	—
	N. I. C.	12.00	29.00**	33.33**	30.33	—	—	—	—	—
	No treatment	7.67	12.00	19.33	23.67	—	—	—	—	—
Gibson, from Tunica, Miss.	Arasan	30.33**	33.00**	33.33**	28.67	—	—	—	—	—
	Spergon	16.33	25.00**	28.67**	29.33	—	—	—	—	—
	N. I. C.	16.33	21.33*	31.33**	28.00	—	—	—	—	—
	No treatment	11.00	14.33	13.67	23.67	—	—	—	—	—
42–91, from Raleigh, N. C.	Arasan	37.67**	38.33**	36.00	33.00	0.33	10.67	29.33**	30.33**	65.75**
	Spergon	31.33**	37.67**	39.00	33.67	1.33	11.00	24.33*	24.67*	71.25**
	N. I. C.	36.33**	39.00**	34.00	31.00	1.33	17.33**	29.00**	19.00	65.50**
	No treatment	18.33	27.00	32.67	35.00	0.67	7.00	15.67	17.00	54.00
42–53, from Raleigh, N. C.	Arasan	40.33**	41.33**	41.67**	36.67	—	—	—	—	75.25
	Spergon	37.00**	42.33***	42.33***	35.33	—	—	—	—	72.75
	N. I. C.	40.33**	38.33**	40.33*	36.67	—	—	—	—	69.50*
	No treatment	25.00	27.67	31.33	33.00	—	—	—	—	61.25
Chief (C), from Illinois	Arasan	32.33	34.00	38.67	41.00	3.00	12.33	15.00	12.67	—
	Spergon	37.33*	38.33*	39.67	35.00	4.00	13.67	15.67	17.33	—
	N. I. C.	35.00	39.33**	38.00	34.33	5.00	10.33	12.67	10.67	—
	No treatment	29.00	29.33	35.67	37.33	1.33	13.00	9.67	10.00	—

a All seed lots were harvested in 1944 except Creole which was 1943 seed.
b Planted 2 days to 3 weeks after treatment.
c Planted 1 year after treatment.
d Planted in field 15 months after treatment.
* Significant at the 5 per cent level (6.87 required for I-A, 7.59 for I-B, 8.00 for I-C).
** Significant at the 1 per cent level (9.04 required for I-A, 10.01 for I-B, 10.65 for I-C).

TABLE 4.—*Effect of seed discoloration, soil temperature, and seed treatment on emergence in Wood's Extra Early Yellow soybean (Series II–A)*

Class	Seed treatment	Average number of seedlings emerged from 50 seeds at		
		20° C.	25° C.	30° C.
1. At least half discolored	Arasan	19.33*	19.00**	20.00**
	Spergon	12.00	11.00	9.00
	N. I. Ceresan	13.00	14.00	4.67
	No treatment	9.67	7.33	2.33
2. Less than half discolored	Arasan	28.33**	28.33**	31.00**
	Spergon	18.67	22.67**	17.33**
	N. I. Ceresan	18.00	15.67*	5.67
	No treatment	12.33	7.33	.67
3. No discoloration	Arasan	25.67**	29.33**	22.00**
	Spergon	18.33	21.67**	11.33**
	N. I. Ceresan	21.33	22.67**	8.67**
	No treatment	14.33	4.00	1.00

* Significant at 5 per cent level (7.64 required).
** Significant at 1 per cent level (10.17 required).

had a significant F value. When the seedling counts from all temperatures were combined and analyzed for variance, those from the treated seed showed significant increases over the checks for most varieties and treatments. The only exception was Chief (C) for which Arasan and New Improved Ceresan did not improve stand significantly.

In Series 1–C, planted in the field (Table 3), seedling emergence was significantly improved by all three fungicides in all five seed lots except Lincoln. The difference between the average counts of seedlings from treated and untreated seed of the other four lots was significant at the one per cent level, except in seed lot 42–53, treated with New Improved Ceresan, in which the difference was significant only at the 5 per cent level.

In Series II, in which Wood's Extra Early Yellow had been separated into three classes, it was expected that the more discolored the seed lot the lower the germination would be, and the greater the improvement from seed treatment. The data on emergence in Series II–A (Table 4) show that in general the more discolored the seed lot the lower the emergence but, con-

TABLE 5.—*Analysis of variance applied to data on emergence in Wood's Extra Early Yellow soybean combining the results from the 3 temperatures (Series II–A)*

Class	Average number of seedlings emerged from 50 seeds treated with			
	Arasan	Spergon	New Improved Ceresan	No treatment
1. At least half discolored	19.44**	10.67	7.22	6.44
2. Less than half discolored	29.22**	19.56**	13.11**	6.78
3. No discoloration	25.67**	17.11**	17.56**	6.44

* Significant at 5 per cent level (4.41 required).
** Significant at 1 per cent level (5.87 required).

trary to expectation, the less the improvement from seed treatment. In Class 1 (Table 5) in which the poorest stand was produced, Arasan was the only treatment that significantly improved emergence, and it was also the most effective treatment for the other classes. More improvement from treatment was obtained at 25° C. and 30° C. than at 20° C. (Table 6).

There was a significant difference in the germination of varieties at different temperatures, and the interactions of varieties × treatments and temperatures × treatments were significant. There was no significant difference between replications and the interaction of variety × temperature did not have a significant F value.

TABLE 6.—*Effect of fungicides at 3 temperatures on emergence in Wood's Extra Early Yellow soybeans combining the results of all seed lots in Series II–A*

Treatment	Average number of seedlings emerged from 50 seeds at		
	20° C.	25° C.	30° C.
Arasan	24.44**	25.55**	24.33**
Spergon	16.33	18.45**	12.55**
New Improved Ceresan	17.43*	17.45**	6.34*
No treatment	12.11	6.22	1.33

* Significant at 5 per cent level (4.41 required).
** Significant at 1 per cent level (5.87 required).

In Series II–B, planted in the field, there was no significant difference in emergence between the controls that were left untreated and the treated seed lots, and there was no significant difference between treatments.

SUMMARY

Temperature had a marked effect on the response of several lots of soybeans to seed treatment. Increases in germination from seed treatment were obtained more often at 25° C. than at any other temperature.

All three fungicides used usually improved emergence from the different seed lots, but Arasan improved it more frequently than either Spergon or New Improved Ceresan.

Seed obtained from Georgia, Mississippi, North Carolina, Maryland, and Virginia appeared to be benefited more by seed treatment than did seed produced in Illinois.

Soybean seed treated and then stored for one year gave a relatively greater increase in emergence over the check than did the treated seed planted soon after treatment, but total emergence was less after storage.

The moderately discolored and the non-discolored seed lots of Wood's Extra Early Yellow soybeans showed greater response to seed treatment than did the badly discolored seed lot, when grown in the constant temperature rooms. When grown in the field, however, there was no significant difference between the reactions of the three lots of seed.

PLANT INDUSTRY STATION,
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THE RELATION OF SIX GROUPS OF FUNGI TO SEEDLING DISEASES OF SUGAR BEETS IN MONTANA[1]

M . M . A F A N A S I E V

INTRODUCTION

Seedling diseases or root rots are of considerable importance in the growing of sugar beets, especially in the heavy irrigated soils in Montana. Numerous investigations have been made on these diseases to determine their nature and control in Montana (1, 2, 5) and elsewhere (3). Pathogenic fungi have been isolated many times, and have been classified in the following genera: *Phoma, Macrosporium, Fusarium, Rhizoctonia, Aphanomyces,* and *Pythium.*

MATERIALS AND METHODS

For isolating *Phoma, Macrosporium, Fusarium,* and *Rhizoctonia* potato-dextrose agar (20 gm. agar, 20 gm. dextrose, and 200 gm. sliced potatoes per liter of water) was used. Diseased beet seedlings were thoroughly washed in tap water, the diseased portions of the roots were cut off and washed in several changes of sterilized water, and then small portions of the diseased roots were imbedded in potato-dextrose agar in Petri dishes. Potato-dextrose agar is a very rich medium and permits luxuriant growth of saprophytic and weakly pathogenic organisms, therefore many of them were isolated by this method.

Cornmeal agar was used for isolating Phycomycetous fungi, suspected of being responsible for seedling diseases of beets. Diseased beet seedlings were thoroughly washed in tap water and the diseased portions of the roots were cut off and placed in sterile Petri dishes filled with sterile water. The beet roots were kept in the Petri dishes for about 24 hours, during which period the sterile water was changed several times. The tissue culture isolations were made on cornmeal agar (15 gm. agar, 20 gm. cornmeal per liter of distilled water).

As soon as the fungal growth appeared it was examined, and very often other kinds of fungi and bacteria occurred with Phycomycetous fungi. The separation of Phycomycetous fungi from other kinds of fungi was made by successive transfers of high dilutions of mixed cultures to cornmeal agar. To separate Phycomycetous fungi from bacteria the following technic was used: High dilutions of organisms were made on cornmeal agar and as soon as fungus colonies appeared, the plates were put in the refrigerator at 5° C. where the fungi continued to grow but bacterial growth was suppressed. Transfers from the edges of the colonies gave fungal growth free of contaminating bacteria. Kauffman's beef-gelatin (4) was also used in separating Phycomycetous fungi from bacteria and the other fungi.

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper No. 191, Journal Series.

One hundred and eight isolates of different fungi were accumulated, and 85 of these were tested for pathogenicity in the greenhouse.

Soil for the pathogenicity studies was obtained from the Huntley Branch Station, Huntley, Montana, and was disinfected with chloropicrin. This soil can be considered typical of the majority of beet soils of Eastern Montana. Duplicate jars of soil were inoculated with each of the cultures of *Phoma, Macrosporium, Fusarium,* or *Rhizoctonia* grown on steamed rice. Two or more jars of soil were not inoculated and were used as checks for each group of organisms tested. Fifteen sugar-beet seedballs treated with New Improved Ceresan (1 oz. for 20 lb. seed) were planted in each jar of soil.

In testing the pathogenicity of reisolated cultures of these fungi, the soil was sterilized in the autoclave and the inoculum was grown in Petri dishes on potato-dextrose agar.

The pathogenicity of the original and reisolated cultures of *Aphanomyces* and *Pythium* was tested in soil sterilized in an autoclave. Two clay pots of soil were inoculated with each of the isolates grown in Petri dishes on corn-meal agar. Two uninoculated pots of soil for each group of organisms were used as checks. Ten sugar-beet seedballs treated with New Improved Ceresan were planted in each pot.

The beets were grown in all these tests for about one month, and healthy and diseased seedlings were recorded at regular intervals. At the end of this period the beet seedlings were dug, washed, and final readings were made.

EXPERIMENTAL RESULTS

In these tests thirteen cultures of *Phoma,* ten cultures of *Macrosporium,* forty-seven cultures of *Fusarium,* five cultures of *Rhizoctonia,* eight cultures of *Aphanomyces cochlioides,* and two cultures of *Pythium* were tested for pathogenicity (Table 1).

The beets in the check pots used for plants inoculated with cultures of *Phoma, Macrosporium, Aphanomyces cochlioides,* and *Pythium* remained healthy. A few of the check plants became diseased in the *Fusarium* and *Rhizoctonia* series. This was probably due to accidental contamination or to the partial sterilization of the soil with chloropicrin. In all of the series where steam sterilization of the soil was used the checks remained healthy.

Four reisolated cultures of *Phoma, Macrosporium,* and *Fusarium,* one of *Rhizoctonia,* eight of *Aphanomyces cochlioides,* and two of *Pythium* were tested for pathogenicity (Table 1). The beets grown in the checks for these tests remained healthy.

The number of emerged plants in all inoculated and check pots for all isolated and reisolated fungi was about the same with the exception of those inoculated with *Phoma,* which indicated that there was very little pre-emergence killing of the plants. Beets grown in pots inoculated with *Phoma* had a much lower stand than in the checks, indicating that there probably was considerable pre-emergence killing.

At the time of harvest many diseased beet seedlings infected with any of these fungi showed a tendency to recover by sloughing off the infected cortical layer of the hypocotyl.

Symptoms of Disease and Degree of Pathogenicity of the Fungi Used in These Tests

Species of Phoma

All cultures of *Phoma* were highly pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 89.1 per cent, but this percentage varied from 33.4 to 100.0 for individual cultures (Table 1). The degree of infection was variable, some beets were infected severely and others only slightly. A culture of *Phoma betae* (Oud) Fr. obtained from the American Type Culture Collection, was also tested for pathogenicity in a

TABLE 1.—*Pathogenicity test of six groups of fungi on sugar-beet seedlings*

Fungus	Original isolates	Reiso-lates	Percentage[a] of seedlings diseased			Pathogen-icity rating
			Min.	Max.	Av.	
	Number	*Number*				
Phoma	13		33.4	100.0	89.1	Medium
Do		4	2.2	71.3	25.3	Variable
Macrosporium	10		6.4	40.9	21.8	Slight
Do		4	0.0	6.6	4.1	Slight
Fusarium	47		3.8	95.5	14.9	Slight
Do		4	tr.	9.4	4.0	Slight
Rhizoctonia	5		8.0	28.5	18.9	Slight
Do		1			20.0	Slight
Aphanomyces cochlioides	8		95.0	100.0	99.4	Severe
Do		8	93.4	100.0	98.6	Severe
Pythium	2		82.3	87.3	84.8	Medium
Do		2	5.3	12.4	8.8	Slight

[a] Minimum and maximum of diseased beet seedlings produced by any one of the cultures. Average of diseased beet seedlings produced by all cultures.

comparison with isolations in Montana. This culture proved to be only slightly pathogenic to sugar-beet seedlings.

The total stand of beet seedlings varied considerably for the different cultures tested. On young infected plants that were not killed before emergence the symptoms of disease usually appeared at time of emergence or soon afterwards. The leaves of severely infected seedlings wilted slightly and usually drooped to the ground. These were somewhat dark-green to bluish instead of the light-green of healthy seedlings. These infected plants usually had dark-brown discolorations of the hypocotyls and some of them were girdled. None of the emerged plants died before harvest. Besides this severe type of infection, numerous plants had slight infection of the underground parts of hypocotyls and roots which could not be detected before harvest. These infections were either brownish discolorations or

lesions. Except for a slight stunting, such seedlings externally appeared to be normal plants.

Four reisolated cultures of *Phoma* were tested for pathogenicity (Table 1). One was very slightly, two were slightly, and one was severely pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 25.3 per cent, but this percentage varied from 2.2 to 71.3 for individual cultures. The symptoms and the development of the disease were similar to those produced by the original cultures.

In conclusion, all isolated and reisolated cultures of *Phoma* proved to be pathogenic to beets and the degree of pathogenicity varied for different cultures.

Species of Macrosporium

Only four cultures of *Macrosporium* produced visible symptoms of disease before the seedlings were dug (Table 1). These diseased plants had yellowing and slight browning of their hypocotyls and some of them were girdled. At harvest time diseased seedlings were found in all sets and their average was 21.8 per cent, but this percentage varied from 6.4 to 40.9 for individual cultures. All diseased roots had slight yellowish-brown discoloration and this was either uniformly present all over the roots or was confined only to certain localized areas. Those plants which had discoloration above the ground were slightly stunted in growth, the remaining plants appeared to be normal. None of the infected plants died before harvest.

Four reisolated cultures of *Macrosporium* were tested for pathogenicity (Table 1). One culture was nonpathogenic and the remaining three only slightly pathogenic. The average number of diseased seedlings was 4.1 per cent, but this percentage varied from 0.0 to 6.6 for individual cultures. The symptoms and the development of the disease were similar to those produced by the original cultures.

The degree of infection for all isolated and reisolated cultures of *Macrosporium* was slight and none of the infected beets died before harvest.

In conclusion, all isolated and practically all reisolated cultures of *Macrosporium* proved to be only slightly pathogenic to beets, and the degree of pathogenicity varied for the different cultures.

Species of Fusarium

All cultures of *Fusarium* were not tested for pathogenicity at once but in several separate tests and all were found to be pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 14.9 per cent, but this percentage varied from 3.8 to 95.5 for individual cultures (Table 1). Diseased seedlings usually showed only light-brown discoloration of their roots. With a few cultures, however, the roots had dark-brown to black discoloration. Also, a few cultures developed a light-brown or black discoloration at the place of seed attachment. One of the cultures produced a dark-brown discoloration of the hypocotyl. Although the infection in

general was slight, in a few jars several infected beets died at the beginning of the experiment.

All four reisolated cultures of *Fusarium* which were tested for pathogenicity (Table 1) were slightly pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 4.0 per cent, but this percentage varied from a trace to 9.4 for individual cultures. The symptoms and the development of the disease were similar to those produced by the original cultures. None of the infected plants died before harvest. In general, the isolated and reisolated cultures of *Fusarium* proved to be slightly pathogenic to beet seedlings, although the percentage of infection was high for some of the cultures.

Species of Rhizoctonia

All cultures of *Rhizoctonia* were pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 18.9 per cent, but this percentage varied from 8.0 to 28.5 for individual cultures (Table 1). All infected plants had a light brown discoloration of the lower parts of their roots. The infection was slight and not a single infected beet died before harvest.

One reisolated culture of *Rhizoctonia* was tested for pathogenicity (Table 1). Twenty per cent of seedlings were diseased and they had dark lesions on all parts of the roots. Some of the roots were girdled and the lower part of the root was lost. Many of the seedlings had remnants of dark infected regions of the hypocotyls immediately below the crown and traces of infection on their roots. Many of this kind of beet were classified as healthy. The degree of infection was slight to medium and several infected beets died before harvest. The symptoms of disease as they are described for reisolated cultures are usually associated with *Rhizoctonia* infection of beet seedlings in field.

In conclusion, all isolated and reisolated cultures of *Rhizoctonia* proved to be pathogenic to sugar-beet seedlings. The degree of pathogenicity varied with different cultures.

Aphanomyces cochlioides Drechs.

Practically all beets inoculated with eight cultures of *Aphanomyces cochlioides* became diseased, the average number of diseased seedlings being 99.4 per cent, but this percentage varied from 95.0 to 100.0 for individual cultures (Table 1). The type of disease produced on sugar-beet seedlings was rather uniform and resembled very closely the "black-root" type of seedling disease occurring in the field. Hypocotyls of practically all diseased seedlings were completely discolored, dark-brown to black, and only a few diseased plants still had remains of healthy hypocotyl tissue. Hypocotyls always had more pronounced discolorations than the roots. This indicates that the disease probably starts either at the surface of the ground or only slightly below it and progresses more upward than downward. The bases of some of the lower leaves were also slightly affected, showing a slight

dark-brown discoloration, but all the leaves remained green and turgid. The diseased plants, stunted in growth and with their stems dark-brown to black and dry, retained their green leaves and turgidity for a long time. There was considerable mortality of the diseased plants before harvest, although on an average more than 50 per cent survived. Infection in most cases was severe.

Practically all beets inoculated with eight reisolated cultures of *Aphanomyces cochlioides* became diseased. The average number of diseased seedlings was 98.6 per cent, but this percentage varied from 93.4 to 100.0 for individual cultures (Table 1). The progress of the disease and the symptoms were exactly the same as for the original cultures.

In conclusion, all isolated and reisolated cultures of *Aphanomyces cochlioides* proved to be highly pathogenic to sugar-beet seedlings.

Species of Pythium

Both cultures of *Pythium* developed a considerable amount of disease. The average number of diseased seedlings was 84.8 per cent, but this percentage varied from 82.3 to 87.3 for individual cultures (Table 1).

During the progress of the disease, before any visible discoloration of the hypocotyls was noticeable, the leaves of diseased beets wilted and drooped. Later, diseased plants became stunted and chlorotic (lemon color) and eventually wilted, and many of them died. Most of the seedlings had a light-brown discoloration of their roots at harvest time. Less than half of the diseased seedlings died before harvest, and the remainder had only a mild degree of infection. Apparently the infection occurred either at the surface of the ground or below it and the injury was confined almost entirely to the roots.

At the time the reisolated cultures of *Pythium* were tested for pathogenicity only a few plants became diseased. The average number of diseased seedlings was 8.8 per cent, but this percentage varied from 5.3 to 12.4 for individual cultures (Table 1). In general, the infection was slight and the symptoms of diseased seedlings closely resembled those inoculated with the original cultures, except that very little yellow discoloration was produced on the hypocotyls of diseased seedlings. Only one diseased plant died before harvest.

In conclusion, the original isolates of *Pythium* were more pathogenic to beet seedlings than reisolated cultures.

DISCUSSION

Results are presented in which beet seedlings were artificially inoculated with six different groups of fungi.

In evaluating these organisms as possible causal agents of seedling diseases of sugar-beets it appears that the symptoms of diseased seedlings, artificially inoculated with *Aphanomyces cochlioides* resembled most closely the majority of beets naturally infected in fields in Montana.

The symptoms of diseased plants artificially inoculated with *Phoma* resembled to some extent the ones infected with *Aphanomyces cochlioides*, except that the leaves of *Phoma*-infected plants showed some wilting; while those infected with *Aphanomyces cochlioides* showed practically none. Also there was a considerable amount of pre-emergence killing of the plants inoculated with *Phoma* and practically none in those inoculated with *Aphanomyces cochlioides*. Undoubtedly every year some beet seedlings are infected with *Phoma* in the field, although the percentage probably is not great.

The symptoms of diseased seedlings artificially inoculated with *Pythium* are typical of damping-off disease and were not observed to any great extent in diseased beets under field conditions. It is believed that *Pythium* has only a minor significance in seedling diseases of sugar beets in Montana.

The pathological symptoms of beets infected by *Macrosporium* and *Fusarium* are not very distinct and for this reason it is difficult under field conditions to diagnose the causal factor of a disease on the basis of the symptoms alone. These symptoms may be confused with the early stages of disease produced by either *Aphanomyces cochlioides* or *Phoma*.

Under the conditions of artificial inoculation, which are very conducive to infection, fungi like *Fusarium* and *Macrosporium* proved to be only slightly pathogenic to beet seedlings. Under competitive field conditions their pathogenicity will be even less. They probably act only as secondary invaders.

The symptoms of diseased seedlings artificially inoculated with *Rhizoctonia* are typical for this disease and beets with this type of symptoms always could be found in a small number in the field.

It appears that seedling diseases of sugar beets from the standpoint of the organisms involved are complex in nature, but under Montana conditions, *Aphanomyces cochlioides* is responsible for more seedling diseases of sugar beets than any other of the above mentioned fungi.

A high percentage of artificially inoculated beets survived. This condition is usually observed in the field. If diseased beets survive until they are approximately in a 6th leaf stage, their recovery usually is assured. Since the number of young beet seedlings present in the field before thinning is always several times greater than that which is required for a proper stand, it appears that even with a bad case of seedling disease it is possible to obtain more or less a normal stand by using delayed, selective thinning. However, the damage to the crop usually results not so much from the reduced stand, as from the retarded growth of plants of low vigor. It is generally true that with a comparatively good stand of beets in the field, in which there was an abundance of seedling disease in the spring, the yields are usually poor because of small beets.

In Montana, seedling diseases of beets can be effectively controlled by improving the physical conditions of the soil and by providing sufficient and

balanced fertilization (1, 2, 5). This usually results in a good stand, rapid and vigorous development of young beets, and high yields.

SUMMARY

1. Numerous isolations of different fungi were made from diseased sugar-beet seedlings in Montana.

2. Pathogenicity of 13 isolated cultures of *Phoma*, 10 of *Macrosporium*, 47 of *Fusarium*, five of *Rhizoctonia*, eight of *Aphanomyces cochlioides*, and two of *Pythium* was tested on sugar-beet seedlings.

3. *Fusarium*, *Macrosporium*, and *Rhizoctonia* were only slightly pathogenic, *Phoma* and *Pythium* were moderately so, and *Aphanomyces cochlioides* was the most pathogenic on sugar-beet seedlings.

4. The symptoms produced by *Aphanomyces cochlioides* on artificially inoculated beets resemble most closely those manifested by the diseased seedlings in the field.

5. Although seedling diseases of sugar beets from the standpoint of the organisms involved are complex in nature, it is believed that *Aphanomyces cochlioides* is responsible for most of the disease in Montana.

6. It is evident that some of the fungi studied are only weak parasites, and possibly they act as secondary invaders.

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KENTUCKY WONDER BEAN PLANTS AS HOSTS FOR MEASURING SOUTHERN BEAN MOSAIC VIRUS ACTIVITY[1]

W. C. PRICE AND BETTY R. HOLT

The Early Golden Cluster variety of garden bean (*Phaseolus vulgaris* L.) was first used for virus activity measurements in 1930 (6). Since then it frequently has been applied in studies on a number of different viruses. A recently described method for estimating the relative activity of Southern bean mosaic virus (*Marmor laesiofaciens* Z. et H.) with errors usually less than 10 per cent makes use of the necrotic local lesions produced by the virus on Early Golden Cluster bean plants (7). Unfortunately, seed of this variety has not been obtainable from commercial houses during the past two years. It has therefore been necessary to make use of another variety of *P. vulgaris* in carrying out activity studies on Southern bean mosaic virus. This paper describes the use of Kentucky Wonder bean plants for such activity measurements.

Most varieties of *Phaseolus vulgaris* respond to infection with Southern bean mosaic virus either by the production of necrotic local lesions or of systemic mottling (10). Symptom expression is controlled by a single pair of allelomorphic factors, localization being dominant to systemic mottling (9). Zaumeyer and Harter (10) reported that the Kentucky Wonder varieties carried the dominant gene for the local lesion response. These varieties were therefore tested for their suitability for activity measurements of Southern bean mosaic virus.

Plants were grown 6 to a pot in 4-inch pots in composted soil in a greenhouse and thinned out to 3 before being used. When the primary leaves reached about three-quarters their mature size they were inoculated with one or another of various dilutions of Southern bean mosaic virus. The virus used for inoculation was obtained from three-weeks infected plants of the Bountiful variety by grinding in a food chopper and expressing the sap through a layer of cheesecloth. Dilutions of the sap were made in appropriate volumes of 0.1 M potassium phosphate buffer at pH 7.

Both Kentucky Wonder Wax and Kentucky Wonder Green Pod leaves developed typical necrotic lesions after inoculation. Table 1 summarizes the numbers of lesions produced on 36 leaves of each of the two varieties with each of 6 concentrations of Southern bean mosaic virus.

The nature of the virus dilution curve has been considered by several workers (1, 3, 5, 8). Recently, Lauffer and Price (4) reviewed the literature on this subject and reached the conclusion that the most tenable hypothesis for explaining the character of the curve is the simple hypothesis that the probability of obtaining infection is related to the probability of find-

ing a single infectious unit in an elemental volume of solution that comes into intimate contact with a susceptible region of the host surface. If y represents the number of local lesions produced with a dilution x of a solution containing n particles per cc., v an elemental volume coming into contact with a susceptible region of the host surface, and N the number of susceptible regions, then

$$y = N(1 - e^{-vnx}) \qquad (1)$$

This equation was applied to virus dilution curve data by Youden, Beale, and Guthrie (8) and independently by Bald (1). If the particles

TABLE 1.—*Total numbers of lesions produced on 36 leaves of Kentucky Wonder Wax and Kentucky Wonder Green Pod bean plants with each of 6 concentrations of Southern bean mosaic virus*

Log. virus concentration	Kentucky Wonder Wax	Kentucky Wonder Green Pod
0	50,400	39,600
−1	31,320	30,960
−2	25,920	14,400
−3	7,920	2,160
−4	1,980	324
−5	144	36

in the solution are aggregated into groups of 2 or more and if b is taken as the number of aggregates and K is a constant, then

$$y = N(1 - e^{-vbx}) \qquad (2)$$

where

$$vbx = \frac{\sqrt{1 + 4Kvnx} - 1}{2K} \qquad (2a)$$

Equation 2 was developed by Bald (2) when it was observed that many sets of virus dilution data failed to fit equation 1 precisely.

The data of table 1 were fitted to the curve of equation 2 with the following constants: K was 2.0 for both varieties, N was 45,000 for the wax variety and 35,000 for the green pod, while vn was 300 for the wax and 95 for the green pod. In figure 1, log (y/N) is plotted as a function of log (vnx). It will be seen from this figure that the experimental data give an excellent fit to the theoretical curve.

The region of the curve most suitable for virus activity measurements is that between log (vnx) = − 0.25 and log (vnx) = − 2.0 (7). This means that for Kentucky Wonder Wax log (x) should be between − 2.75 and − 4.5 and for Kentucky Wonder Green Pod it should be between − 2.25 and − 4.0. Since both N and vn can be expected to vary from experiment to experiment, due to variation in growing conditions and age of host plants, these limits are also expected to vary. It has been the general practice in this laboratory to prepare the standard virus preparation from freshly expressed sap of diseased Bountiful bean plants at dilutions of 10^{-3} and 10^{-4}. With few exceptions these concentrations can be expected to lie along the curve at

the most favorable points for activity measurements. It can be seen from figure 1 that over this range the log of the number of lesions produced is very nearly a linear function of the log of the virus concentration.

In the experiments summarized in table 1 and figure 1, the same virus preparation was used in inoculating both types of plants. Hence n, the number of virus particles per cc., must have been the same in both dilution series. This means that v, the elemental volume of solution coming into contact with a susceptible region of the leaf surface, was about 3 times as great for the wax variety as for the green pod. From this it can be reasoned that the susceptible area on the wax type is about 3 times as large as that on
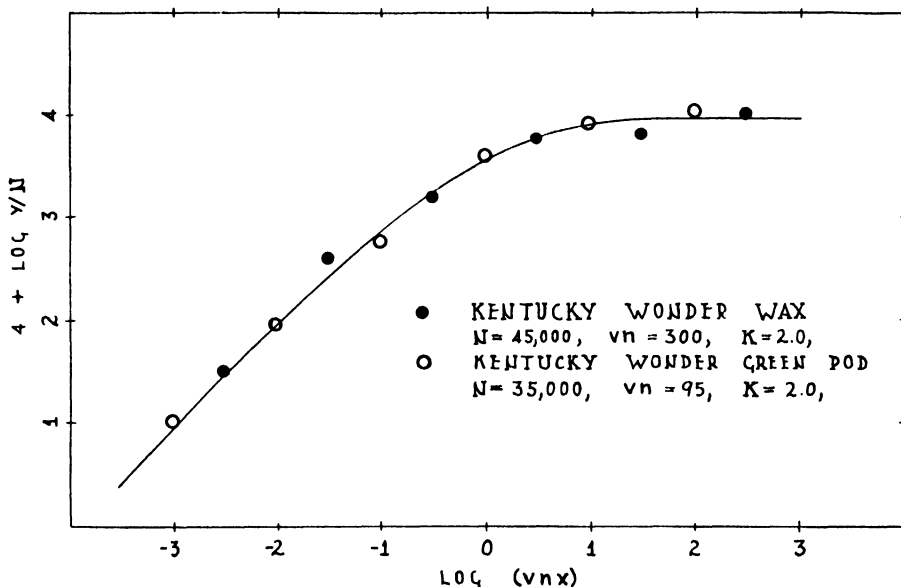
FIG. 1. Graph of Southern bean mosaic virus dilution curve when Kentucky Wonder varieties of bean were used as host plants.

the green pod. Since N was 45,000 for the wax bean and 35,000 for the green pod, and since an equal number of leaves were used in both cases, there were apparently about 28 per cent more susceptible areas on the wax than on the green pod variety.

It is of interest that while the two virus infection series vary with respect to N and v, factors contributed by the host, they do not vary with respect to K. This lack of variation with respect to K is to be expected if K measures the degree of aggregation of virus particles in the virus solution, as was assumed in the derivation of equation 2a. It would be of value to carry out additional dilution curve studies with different preparations of virus to determine whether or not K is a constant for each virus preparation.

Both Kentucky Wonder Wax and Kentucky Wonder Green Pod varieties have been used in this laboratory to measure activities of Southern bean mosaic virus preparations. While there are no data available for determining

precisely the accuracy of these measurements, it appears from general considerations that they are as accurate, if not more accurate, than those involving the Early Golden Cluster variety as a host. In table 2 are presented data from an experiment designed to measure the activity of a virus sample which was expected to have an activity slightly less than that of the standard against which it was tested. When the figures in table 2 were converted to logarithms, the logarithm of the relative activity of the unknown in terms of the standard and its standard error were calculated according to the method previously described (7). The calculated value is −0.182, indicating a relative activity of 66 per cent. The calculated standard error of the log is 0.053. The standard error is of the same order as that found previously when the Early Golden Cluster variety was used as the test plant (7). The standard errors calculated from similar experi-

TABLE 2.—*Numbers of lesions produced on 3 half leaves of Kentucky Wonder Green Pod bean plants with different concentrations of Southern bean mosaic virus*[a]

Pot. No.	S_1	U_1	S_2	U_2
	(L)	(R)	(L)	(R)
1	126	164	393	502
2	105	146	306	267
3	117	147	326	373
4	72	112	270	255
	(R)	(L)	(R)	(L)
5	173	69	463	303
6	102	49	297	306
7	213	63	300	187
8	125	71	343	268

[a] S and U refer to the standard and unknown virus preparations, L and R refer to the left and right halves of the test plant leaves.

ments performed during the last 18 months have been of the same order. From the results mentioned above it can be concluded that either Kentucky Wonder Wax or Kentucky Wonder Green Pod can serve as a suitable substitute for the Early Golden Cluster variety in measuring the activity of Southern bean mosaic virus.

SUMMARY

The numbers of necrotic lesions produced on both Kentucky Wonder Wax and Kentucky Wonder Green Pod bean plants, when inoculated with a series of dilutions of Southern bean mosaic virus, were found to follow an equation derived on the assumption that the virus particles are aggregated and that the chance of obtaining infection is related to the chance of finding a single infectious aggregate, or particle, in a unit volume of inoculum. It is a characteristic of this equation that over a certain range the log of the number of lesions is very nearly a linear function of the log of the virus concentration. It follows that the Kentucky Wonder varieties of bean are suitable test plants for use in the method of measuring Southern bean mosaic

virus activity that involves comparison of two dilutions of the unknown virus preparation with two dilutions of a standard. Experimental data obtained over a period of 18 months, an example of which is given in the text, bear out this conclusion.

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COMPARISON OF TWO METHODS OF PELLETING
ONION SEED IN THE CONTROL OF SMUT

M. B. LINN[1] AND A. G. NEWHALL[2]

A method for pelleting vegetable and other kinds of seed has been developed by the Farmers and Manufacturers Beet Sugar Association, Saginaw, Michigan in cooperation with the Dow Chemical Company, Midland, Michigan. The primary purpose is to add bulk to light seed and to make all individual seeds uniform in size in order to facilitate precision sowing with mechanical equipment. Briefly, the process involves placing the seed in a revolving, spherical-shaped pan, then alternately wetting with a binder (methyl cellulose solution) and dusting with feldspar and other materials until round pellets of the desired dimensions have been formed. Thus fungicides, insecticides, hormones, fertilizers and the like can be built into the "pill" and planted with the seed.

Another method of pelleting seed described by the junior author[3,4,5] in 1944 was developed in New York as a replacement for the laborious formaldehyde drip method of controlling onion smut. It consists of moistening the seed by stirring with a 5 per cent solution of methyl cellulose and then coating with an equal weight of an organic sulfur fungicide, Arasan or Tersan, by agitation for 5 minutes in an electric paint can shaker, or, as the senior author has found satisfactory, by rolling in a barrel or churn. The feldspar excipient is not used in the New York method and the sticker solution is less concentrated.

The pellets resulting from the two methods are somewhat different, as seen in figure 1. The use of feldspar and of repeated applications of a more concentrated sticker solution results in a comparatively large, hard, uniformly spherical pellet which on first sight would appear to be very desirable. In the New York method the pellets come out, not quite spherical, and more or less mealy rather than hard or brittle. However, the New York method is less expensive than the Michigan method and better adapted to the needs of a temporary portable system of custom treatment or of individual farm treatment of seed. Experiments conducted in Illinois and New York comparing Early Yellow Globe onion seed treated by each method were carried on by the writers in 1945 with the idea of evaluating the treatments from the standpoint of smut control, stand of seedlings, and ease of sowing in the conventional type seeders now in use.

Two one-pound samples were pelleted with Arasan and Tersan respectively through the courtesy of Mr. Arthur A. Schupp and Dr. Phelps Vogelsang at Saginaw, by the Beet Sugar Association feldspar method hereafter designated the Michigan method while two more were treated at Cornell by the junior author, one each with Arasan and Tersan. The rate was a pound of fungicide to a pound of seed. One pound was left untreated. The Michigan treated seed was several times larger due to the amount of sticker and excipient employed, in addition to the pound of fungicide.

One portion of each of the above 5 samples of seed was sown in Cook County, Illinois, in a field heavily infested with smut. Hole 20 of the No.
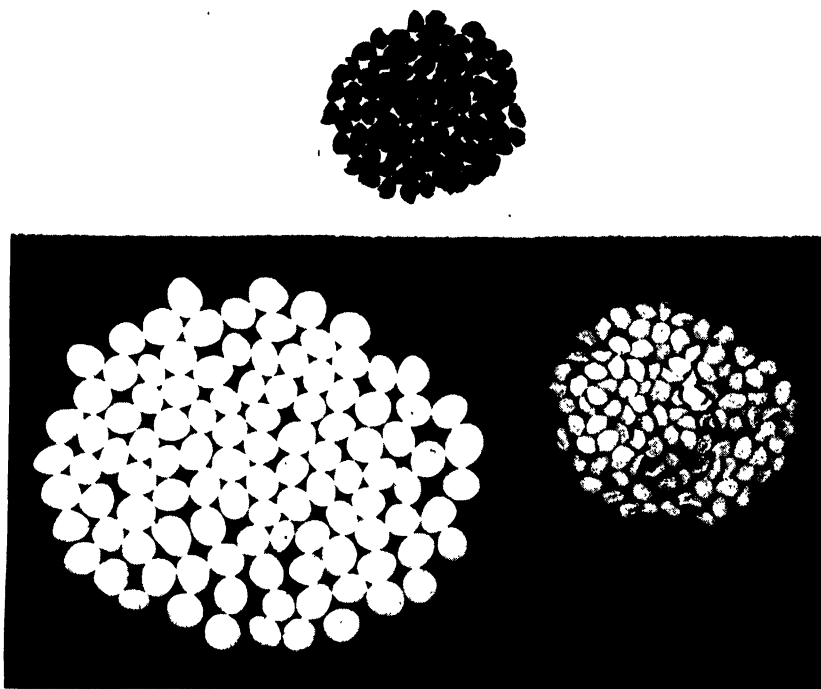
Fig. 1. Untreated onion seed (top), compared with same number pelleted by the Michigan feldspar method (lower left), and by the New York method (lower right).

300 Planet Jr. drill had to be used in sowing the Michigan pelleted seed, hole 10 for the New York pelleted seed, and hole 8 for the untreated in order to sow approximately the same number of seeds per foot. In New York four plantings were made. Two were in the greenhouse and consisted of replicate samples of 100 seeds each. A similar one was made in the field, and a larger test, also in the field, employed an Iron King single row drill on several rows 300 feet long. Deep sowing in Illinois was coupled with excessive rainfall and, therefore, delayed emergence resulted in poor smut control. In the first New York greenhouse test six replications of 100 seeds were sown and although the differences in smut control were slightly in

favor of the New York method over the Michigan method they are not significant at the 5 per cent level. All are significantly better than the check. In the second greenhouse test made in New York an excess of moisture was purposely applied with the result that considerable late infection took place giving high smut counts. In the small New York field test there was so little smut that the significant benefits over the check from all treatments, replicated 5 times, can be attributed chiefly to control of damping off, there being no significant differences in smut control. Maggot injury was so severe in the fourth New York unreplicated field test that no smut or stand counts were made, but the effect of the rotating agitator of the drill on the

TABLE 1.—*Comparison of Michigan feldspar-pelleted onion seed with New York-pelleted seed in the control of smut*

Location of test	Kind of plots	Fungicide	Pelleting process	No. plants emerged and counted	Ave. percentage smutted seedlings[a]
New York	Green-house, 1	Arasan	Michigan	95	10.6
			New York	92	9.6
		Tersan	Michigan	84	14.3
			New York	95	8.4
		None	None	72	51.5
	Green-house, 2	Arasan	Michigan	89	23.0
			New York	82	23.0
		Tersan	Michigan	76	22.0
			New York	81	19.0
		None	None	73	53.0
	Field	Arasan	Michigan	70	3.1
			New York	69	1.4
		Tersan	Michigan	67	0.5
			New York	70	2.7
		None	None	55	7.0
Illinois	Field	Arasan	Michigan	100	70.0
			New York	100	40.0
		Tersan	Michigan	100	78.0
			New York	100	52.0
		None	None	100	94.0

[a] In the New York tests, this represents the average of five replicated plots in the greenhouse and five in the field using 100 seeds in each plot. Plots were unreplicated in the two field tests.

stability of the pellets and seeding rate was closely observed. The results of these tests are summarized in table 1.

It would appear from all of the tests that the Michigan method employing feldspar did not give superior smut control nor better stands than the less expensive New York method, in fact what little difference there was seemed to be slightly in favor of the latter. In these and other tests both writers observed a considerable amount of unavoidable cracking of the feldspar pellets when sown with seeders having metal or wooden based agitators. The longer the rows the more of this occurred and in the Illinois test the poorer control of smut may have been due in part to this destruction of the

pellet. The seeding rate was believed to have slowly increased in one New York trial as time went on and more seeds were freed from their coating.

Since cracking of the pellets was thought to be due in part at least to interfacial friction, tests were run in Illinois to see if this trouble could be corrected by treating pellets with graphite. A fine lubricating graphite was mixed with Michigan feldspar-Tersan pelleted seed at the rate of 1½ ounces to 60 pounds of pellets, which seemed to be the maximum that could be retained. Although the graphite tended to reduce friction a little, the benefit was rather slight as indicated in table 2 and the cracking of pellets and liberation of bare unprotected seeds was not eliminated. Experiments in New York by the junior author likewise resulted in the rejection of graphite for expediting the flow of onion seed mixed with dry Arasan.

TABLE 2.—*Effect of graphite on seeding rate of Michigan feldspar-pelleted onion seed*

Treatment	Number of pellets sown per foot							
	Seeder plate hole number							
	10	12	14	16	18	20	22	24
Graphite	2.6	4.1	5.7	6.1	7.5	12.8[a]	15.3	21.3
No graphite	2.3	3.2	4.4	5.6	7.0	9.5	12.8	19.8

[a] This rate is approximately equivalent to that obtained with New York pelleted seed using hole 11 and with nonpelleted seed using hole 9.

The conclusions reached are that when seed drills are available for onion growers which can handle the tough hard pellets, turned out by the Michigan feldspar method, without cracking, splitting, and crushing a large proportion of them, then the advantages of more even spacing of seeds in the ground may help offset the added expense of treatment by the Michigan method, but until then the advantages from the standpoint of smut control, economy, and even stands are somewhat in favor of the New York method of pelleting.

Both methods may at times result in two or even three seeds occurring in one pellet which can affect yield records. Counts of the samples used in these experiments revealed a greater number of doubles in the Michigan than in the New York pelleted seed (11 per cent vs. 2 per cent) which may be due to the fact that the total amount of seed was small and more difficult to treat perfectly in the Michigan apparatus.

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FIELD STUDIES ON SPREAD OF THE MILD STREAK DISEASE OF BLACK RASPBERRIES

W. F. JEFFERS AND M. W. WOODS[1]

Mild streak, a disease apparently of virus nature, is the most serious trouble affecting black raspberries in Maryland. This disease was first observed in Maryland about 1935 and since that time has increased to such an extent that nearly all plantings in the State are affected.

Loss from mild streak is due to the effect on the fruit. Fruit symptoms vary to some extent in different varieties and there are also seasonal variations. However, typical fruit symptoms consist of loss of gloss and a pitting or shrinking of the individual drupelets. This results in undersized, dull, seedy, unattractive fruit of poor flavor.

Discolored streaks of varying intensity can usually be found on the lower portion of young canes affected with mild streak. This symptom varies greatly with variety and season but generally consists of elongated, water-soaked markings which later in the season become more clearly defined and somewhat purplish. However, in many instances the streaks are so faint as to be barely discernible. Fruit and cane symptoms are shown in figure 1.

Mild streak was first described as being distinct from severe streak by Rankin (6) in 1931, but he had apparently been familiar with this disease for several years previous. In 1932 Cooley (3) presented a complete description of the symptoms of mild streak. Since this time various other workers have presented descriptions of mild streak (1, 2, 4, 5, 7, 8, 9, and 10). Rankin (6) had previously shown that plants within ten feet of mild streak infected plants were much more apt to become infected than those further away. Rankin also proved that mild streak spread more rapidly in the variety Ohio than in Plum Farmer or Cumberland. Cooley (3) stated that the rate of spread is usually slow; about doubling each season. However, in vigorous plantings, the rate was more rapid. Woods and Haut (10) reported the spread of mild streak to be rather rapid up to 20 feet but slower up to 200 feet.

To obtain information leading to possible control measures, a study of mild streak under field conditions was begun. In the spring of 1941 about 2,500 plants of the Cumberland black raspberry were obtained from the Ohio Small Fruit Improvement Association. These plants showed no symptoms of mild streak during the first growing season. The field in which these plants were set had not been previously planted to raspberries but the north side was bordered by a hedge row containing wild black raspberries and blackberries and was about 200 yards from several old plantings containing much mild streak. The east side of the field was about 100 feet

FIG. 1. Symptoms of mild streak disease on fruit (A and B) and cane (C) of the Cumberland black raspberry.

from a woods containing wild brambles. The land sloped rather steeply from the high southwest corner to the low northeast corner.

During the fruiting season of 1942 a careful inspection revealed that 37 of 2,435 plants were infected with mild streak. The infected plants were distributed rather uniformly throughout the field. These plants were burned, in place, with a flame thrower, and later removed. Thus it was apparent that some infection must have occurred during the growing season of 1941 or early in 1942 or that there was some mild streak present in the plants when set. Inspection during the rest of 1942 showed no evidence of

TABLE 1.—*Amount of mild streak in sections of test planting of Cumberland raspberries from 1942 through 1947*

Location	Plants observed	Year	Plants infected with mild streak	
	Number		*Number*	*Per cent*
Section 1	729	1942	12	1.6
Section 2	846		11	1.3
Section 3	860		14	1.6
Total	2435		37	1.5
Section 1	718	1943	417	58.1
Section 2	846		73	8.6
Section 3	860		57	6.6
Total	2424		547	22.6
Section 1	714	1944	481	67.4
Section 2	827		198	23.9
Section 3	852		132	15.5
Total	2393		811	33.9
Section 1	708	1945	586	82.8
Section 2	768		329	42.8
Section 3	832		217	26.1
Total	2308		1132	49.1
Section 1	709	1946	629	88.7
Section 2	704		449	63.8
Section 3	809		320	39.6
Total	2222		1398	62.9
Section 1	700	1947	670	95.7
Section 2	698		660	94.6
Section 3	804		681	84.7
Total	2202		2011	91.3

further infection. In 1943 the distribution of mild streak was again plotted. It was found that 547, or 22.6 per cent, of the 2,424 plants examined were infected. The increase in amount of infection was greatest in the north end of the planting. In view of the large amount of infection and because this planting was in cooperation with a commercial grower, roguing was discontinued.

Observations made as early as May 15, 1943, soon after new growth appeared, showed that the incidence of mild streak was much greater than in 1942. This early appearance of mild streak symptoms together with the fact that no further infected plants were recognized during the remainder of

the 1942 season suggests that current season infection cannot be readily recognized.

Spread of mild streak in different parts of the test planting was determined by dividing the fields into three sections. Section one included twenty rows at the north end; section two, the central twenty rows and section three, the twenty rows on the south end. The amount of mild streak found in each section for the six year period of 1942-1947 is shown in table 1.

The rapid spread of mild streak in section one has not been correlated with any factor but it seems possible that it may be related to the distribution of an insect vector and the proximity of wild and escaped brambles. Cooley (3, 4) and Rankin (6), on the basis of field observations, suggested that mild streak may be transmitted by an insect vector. Bennett (1) tested *Amphorophora rubi, Aphis rubiphila,* and *Amphorophora sensoriata* for their ability to transmit streak but obtained only negative results.

TABLE 2.—*Insects, belonging to groups known to transmit plant viruses, collected in test planting*

Aleyrodinae (2 species) Possibly Trialeurodes	*Erythroneura torella,* Rob.
Aulacaspis rosae (Bouché)	*Erythroneura sp.* Probably *obliqua* (Say)
Graphocephala coccinea (Forst.)	*Amphorophora sp.*
Scaphtopius acutus (Say)	*Cinara sp.*
Lepyronia quadrangularis (Say)	*Macrosiphum ambrosiae* (Thos.)
Empoasca erigeron, Del.	*Macrosiphum pisi* (Kalt.)
Empoasca fabae (Harris)	*Myzocallis punctatellus* (Fitch.)
Empoasca copula, Del.	*Cercosipha rubifolii* (Thos.)

Observations of insects present in this planting showed a stem-inhabiting aphid, tentatively identified as *Amphorophora sensoriata* Mason, to be the most prevalent aphid species present. Sweepings yielded a number of other insects. Those belonging to groups known to transmit plant viruses are listed in table 2.[2]

Of the various insects listed in table 2, only *Cercosipha rubifolii* (Thos.) is known to feed on *Rubus.* While the possibility that one of these or some other insect may transmit mild streak cannot be excluded it is felt that circumstantial evidence warrants further testing of *Amphorophora sensoriata.* Transmission experiments with this insect are now in progress.

SUMMARY

1. Mild streak, a disease apparently of virus nature, is widespread in Maryland black raspberry plantings.

2. Rapid spread of the disease may occur under field conditions.

3. Current season infection cannot be readily recognized.

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[2] The authors are indebted to the U. S. National Museum for identification of these insects.

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RESISTANCE TO BACTERIAL WILT AND BLACK SHANK IN FLUE–CURED TOBACCO[1]

T. E. SMITH AND E. E. CLAYTON

INTRODUCTION

Bacterial wilt (*Bacterium solanacearum* E. F. Smith) and black shank (*Phytophthora parasitica* var. *nicotianae* Tucker) have caused serious diseases of tobacco for many years in restricted areas of the southeastern United States. Wilt occurred in North Carolina in the north central and eastern sections. Black shank occurred in the shade tobacco section of Florida and Georgia and in the flue-cured tobacco section of North Carolina and Virginia. Separate flue-cured tobacco varieties resistant to each disease have been developed and introduced. T.I. 448A from Colombia, South America, was shown to have high resistance to wilt (3) and in later work, Oxford 26, a resistant flue-cured variety, was selected from the cross of T.I. 448A × 400 (5). Florida 301 (6) was highly resistant to black shank in North Carolina and it was used in crosses with flue-cured varieties to develop Oxford 1, 2, 3, and 4 (2). These new varieties are grown successfully on soils infected with either bacterial wilt or black shank. However, in recent years black shank has spread into regions where wilt occurred. The situation now requires a variety highly resistant to both diseases. This paper is a report of resistance to black shank in genotypes resistant to bacterial wilt and of resistance to bacterial wilt in genotypes resistant to black shank. Results are also reported from a cross between wilt and black shank resistant genotypes.

RESULTS

Resistance to black shank was measured in replicated plantings made on infested field plots. To measure the degree of resistance to black shank, counts of dead or obviously diseased plants were made. Three wilt resistant genotypes (T.I. 448A, Oxford 26, and 43–2) were compared with Florida 301 which is black-shank resistant, and 4 flue-cured varieties (Gold Dollar, 400, 401, and Yellow Special) that are susceptible to both diseases. Approximately 3 months after transplanting, Florida 301 averaged 11 per cent black shank, and T.I. 448A averaged 49 per cent black shank. The difference between Florida 301 and T.I. 448A was significant (0.01). Oxford 26 and 43–2, F_6 lines of T.I. 448A × 400, averaged 86.5 per cent black shank and the four susceptible flue-cured varieties averaged 97.5 per cent black shank. The difference between the F_6 lines and the susceptible varieties was significant (0.01) in an analysis of variance of the transformed (1) data.

Thus all three wilt resistant genotypes tested had some resistance to black shank.

Resistance to bacterial wilt was also measured in replicated plantings on infested field plots. To measure wilt resistance, counts were made of plants dead or obviously wilted on 2 dates. Four black-shank-resistant genotypes (Oxford 1, 2, 3, and 201) were compared with wilt resistant Oxford 26 and two flue-cured varieties (Gold Dollar and 400) that were susceptible to both diseases. In midseason, 2 months after transplanting, all varieties had significantly (0.01) more wilt than Oxford 26. Among the black-shank-resistant genotypes, only Oxford 2 (51 per cent wilt) and Oxford 201 (54 per cent wilt) had significantly (0.01) less wilt than the average of the two susceptible flue-cured varieties (87 per cent). Near the end of the growing season, 4 months after transplanting, Oxford 2 was badly diseased. However, on this date, Oxford 201, an F_5 of Florida $301 \times 400 \times 400 \times 400$, averaged 63 per cent wilt in comparison to 15 per cent in Oxford 26 and 99 per cent in Gold Dollar. Thus, some wilt resistance occurred in 2 of the 4 genotypes resistant to black shank.

Lines resistant to wilt and to black shank were crossed in 1941. The hybrids were selected for wilt resistance and desirable growth characters in the F_2, F_3, and F_4. An F_5 wilt-resistant line, designated as Oxford 202, was grown in separate wilt- and black-shank-infested plots. On the wilt plots, 11 single plant selections of Oxford 202 averaged slightly less wilt than T.I. 448A under conditions where Gold Dollar was 100 per cent wilted. On the black-shank plots, these same lines of Oxford 202 averaged significantly (0.01) less black shank than Gold Dollar. In further tests of black-shank resistance in Oxford 202, nine F_6 lines averaged 47 per cent black shank in a planting where Florida 301 had 3 per cent and 400 had 100 per cent. These results on wilt-infested soil showed that very high resistance to wilt was recovered, and furthermore, many lines selected only for wilt resistance had moderate resistance to black shank.

DISCUSSION AND CONCLUSIONS

Resistance to bacterial wilt in T.I. 448A and resistance to black shank in Florida 301 are inherited on a multiple factor basis (2, 4, 6). Many field-plot data and farm experience have shown that the full resistance of each basic stock is necessary for effective disease control under conditions of continuous tobacco culture. The full resistance was difficult to recover after crossing. For example, in the F_2 of T.I. 448A × flue-cured varieties, 63 segregates of high wilt resistance were selected from a total population of 5200, a ratio of 1:82. In the F_2 of Florida 301 × flue-cured varieties, 29 segregates of high black-shank resistance were selected from a total population of 935, a ratio of 1:31. Based on these field-plot results, the F_2 of a cross between genotypes resistant to wilt and resistant to black shank should carry segregates of high resistance to both diseases in the ratio of 1:2655. In the absence of linkage, the development of a flue-cured variety highly resistant to both diseases would be an extremely difficult undertaking.

Linkage between wilt resistance and black-shank resistance was indicated by the results obtained since 1944. T.I. 448A, the source of resistance to wilt, was moderately resistant to black shank. After a cross of T.I. 448A × 400, selection for wilt resistance isolated F_6 lines with low resistance to black shank. Among black-shank-resistant varieties selected from backcross generations of Florida 301 × flue-cured varieties (recurrent parent), Oxford 2 and 201 had measurable resistance to wilt. Further evidence of linkage was obtained from the cross of a genotype resistant to wilt × a genotype resistant to black shank. The progeny of this cross was selected only for wilt resistance in the F_2, F_3, and F_4. This material, designated as Oxford 202, had high wilt resistance and moderate black-shank resistance in the F_5 and F_6. It is significant that the initial F_2, out of which Oxford 202 was selected, contained only 240 plants or approximately one-eleventh of the calculated number required for recovery of one F_2 segregate with high resistance to both diseases. Linkage increased the number of segregates with combined resistance. The results show that a flue-cured variety of tobacco with high resistance to wilt and black shank can probably be developed without resort to excessively large populations.

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CANNA MOSAIC IN THE UNITED STATES

PHILIP BRIERLEY[1] AND FLOYD F. SMITH[2]

In September, 1942, Mr. Horace V. Wester of the National Capital Parks, Washington, D. C., submitted to the writers three samples of ornamental cannas (*Canna generalis* Bailey) as follows: Richard Wallace showing symptoms of a virus, the same variety apparently healthy, and the variety The President apparently healthy or symptomless. Two plants of *Canna glauca* L.[3] occurred in mixture with the mosaic Wallace, and showed strong yellow stripe symptoms. In December, 1945, Dr. George M. Reed supplied 5 varieties of canna previously selected at the Brooklyn Botanic Garden by Floyd F. Smith. These varieties were Indiana, King Humbert, Richard Wallace, Wyoming, and The President, all but the last showing symptoms of a virus disease. In nurseries in Michigan and Delaware visited by the writers in 1946 and 1947, all of 25 or more canna varieties grown except The President showed the same virus symptoms. We have attempted to determine whether this common mosaic of canna in the United States agrees with the mosaic of *Canna indica* L. previously described from the Philippines,[4] and whether The President is resistant or immune to this virus.

Occurrence of a mosaic of *Canna indica* in Japan was reported by Fukushi[5] in 1932, and by Ocfemia[6] in the Philippines in 1937. A detailed experimental study of the disease was made by Ocfemia, Macaspac, and Yuan,[4] who found it locally abundant in *C. indica* in the province of Davao. These workers describe and illustrate symptoms of canna mosaic in *C. indica* and in abaca (*Musa textilis* Nee). Early leaf symptoms are fine chlorotic lines connecting two branch veins. Later spindle-shape chlorotic areas may appear. In advanced stages continuous or broken chlorotic stripes extend from the midrib to the leaf margin parallel with the veins, and the leaf may become wrinkled or curled. Such stripes may become brown necrotic in late stages, particularly in ornamental cannas. Streaking of the petal color in *C. indica* and in ornamental varieties is also mentioned. Canna mosaic was not seed borne in *C. indica*. One successful transfer by leaf-rubbing was considered inconclusive. Transmission was successful

[3] We are indebted to Dr. F. J. Hermann for identification of the species of *Canna*, *Hedychium*, and *Musa* mentioned in this paper.

[4] Ocfemia, G. O., I. S. Macaspac, and H. F. Yuan. Experimental transmission of the mosaic of *Canna indica*. Philipp. Agric. 30: 357–370. 1941.

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with the aphids *Aphis gossypii* Glover and *A. maidis* Fitch, but not with *A. laburni* Kalt., *Pentalonia nigronervosa* Coq., or *Rhopalosiphum nymphaeae* L. Canna mosaic was transmitted to *C. edulis* Ker. as well as to *M. textilis*, *C. indica*, and ornamental cannas by *A. gossypii*, but this vector failed to transmit the virus to cotton or cucumber. *Aphis gossypii* was shown to transmit canna mosaic virus in the non-persistent manner, acquiring the virus in 5 minutes' feeding on a source plant and becoming non-infective after feeding on one healthy plant or after 1 hour without food.

MATERIALS AND METHODS

The canna varieties listed above served as sources of the virus. The procedures used in mechanical transmission and in insect transfer experiments were the usual ones that we described earlier.[7] Canna seedlings were used as test plants. *Canna glauca* which seemed promising for this purpose, failed to set seed in our cultures even when hand-pollinated. Seed of Richard Wallace open pollinated in the field produced virus-free seedlings, but these proved somewhat inferior for test purposes because of weak symptom expression when infected. In December, 1945, we received seed of *C. indica* from Trinidad, through the courtesy of Mr. B. Y. Morrison of the Division of Plant Exploration and Introduction. These seeds germinated readily after scarifying and produced satisfactory test plants. Test plants of *Hedychium coronarium* Koenig were divisions of individuals purchased from a nursery in Alabama, and divisions of *Musa cavendishii* Lamb. and *M. textilis* were obtained from stock growing in the greenhouses at Plant Industry Station, Beltsville, Maryland.

IDENTITY OF THE VIRUS

There is complete agreement in the symptoms observed in our material of *Canna indica* (Fig. 1, A) and ornamental cannas (Fig. 1, B) with those described by Ocfemia, Macaspac, and Yuan for these species in the Philippines. Similar symptoms occurred in *C. glauca* (Fig. 1, C) with necrotic streaks prominent in the expanded leaves. This species was not included in the Philippine study and their *C. edulis* was not available to us. Repeated inoculation of *Hedychium coronarium*, *Musa textilis*, and *M. cavendishii* by suitable aphid vectors has failed to induce symptoms in these species in our trials, and *Myzus persicae* (Sulz.) also failed to recover the virus from inoculated *M. textilis*. As previously noted, Ocfemia, Macaspac, and Yuan readily infected *M. textilis* in the Philippines. This discrepancy is possibly not significant, as it is doubtful whether our potted *M. textilis* ever attained the vigorous growth that this species makes in the open in the tropics. Canna mosaic was not transmitted to cucumber in two trials, nor *Vicia faba* L. in one trial. In comparative tests *M. persicae* transmitted cucumber mosaic to cucumber and to *C. indica*, but transferred canna

[7] Smith, Floyd F., and Philip Brierley. Ornithogalum mosaic. Phytopath. **34:** 497–503. 1944.
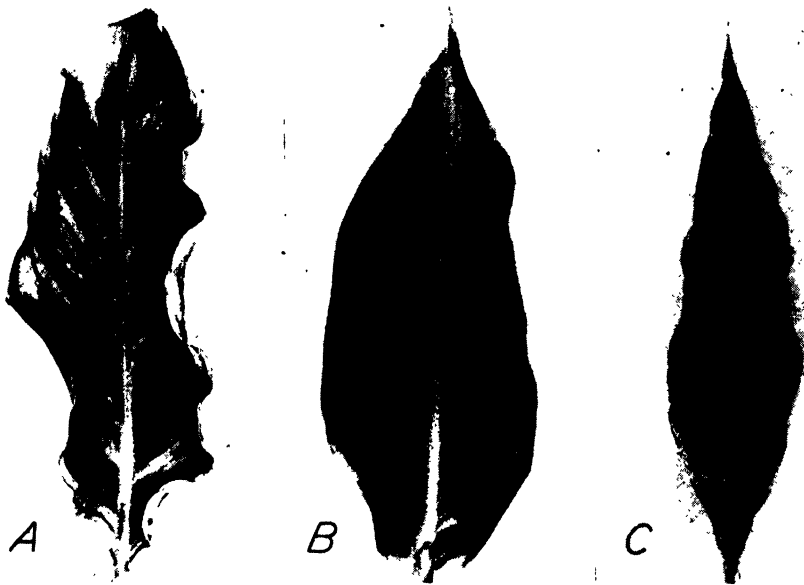
FIG. 1. Symptoms of canna mosaic. A. *Canna indica;* B. Ornamental canna variety Richard Wallace; C. *Canna glauca.* Photos by O. H. Greeson.

mosaic to *C. indica* only. Cucumber mosaic was reisolated mechanically in tobacco from cucumber and from *C. indica,* but no transfer of canna mosaic from *C. indica* resulted in a parallel test on tobacco. Initial symptoms of canna mosaic in *C. indica* have been noted 4 times after intervals

TABLE 1.– *Transmission of canna mosaic by aphids*

No. of trials	Source of virus	Plants inoculated	Plants affected
Aphis gossypii			
2	Canna glauca	C. generalis	0/15
1	Canna glauca	C. indica	0/3
1	Richard Wallace	C. indica	1/5
Aphis maidis			
2	C. glauca	C. generalis	7/15
Macrosiphum solanifolii			
1	Richard Wallace	C. indica	2/5
1	C. glauca	C. indica	0/5
Myzus circumflexus			
1	C. glauca	C. indica	1/5
Myzus convolvuli			
1	Richard Wallace	C. indica	0/5
Myzus persicae			
2	C. glauca	C. generalis	4/13
1	C. glauca	C. indica	0/2
3	C. indica	C. indica	10/12
1	King Humbert	C. indica	4/5
2	The President	C. indica	0/8
4	Richard Wallace	C. indica	15/20
1	Richard Wallace	The President	0/2
2	Wyoming	C. indica	5/9

of 13 to 15 days, but in many plants symptoms were first detected after 20 to 40 days. This is good agreement with the Philippine report of 2 weeks stated by Ocfemia, Macaspac, and Yuan.

No evidence of seed transmission was detected when 44 seedling *Canna indica* were grown from seed of mosaic plants; Ocfemia, Macaspac, and Yuan also report no seed transmission. Mechanical transmission by the carborundum leaf-rubbing method failed on 10 *C. indica* plants in one trial, but produced typical canna mosaic symptoms in 4 of 25 plants inoculated in later trial from *C. indica* to more succulent test material of *C. indica*. Ocfemia, Macaspac, and Yuan reported one mechanical transfer to *Musa textilis*, which they considered of doubtful significance.

Aphid transmission tests in cannas are summarized in table 1. All tests were conducted with the technique suited to nonpersistent viruses, the successful transfers, therefore, confirming the nonpersistent nature of canna mosaic virus which the Philippine workers had previously reported. Transmission by *Aphis gossypii* and by *A. maidis*, previously shown to be vectors, was successful. However, *A. gossypii* was comparatively inefficient as a vector in our trials, although very efficient in the Philippines. Three additional vectors, *Macrosiphum solanifolii* (Ashm.), *Myzus circumflexus* (Buckt.), and *M. persicae* were established, but *M. convolvuli* (Kltb.) failed to transmit canna mosaic in a single trial. *Myzus persicae* proved to be the most efficient of the species used in our trials, and was, therefore, used in most of our attempts to establish additional hosts outside the genus *Canna*, all of which were unsuccessful.

IMMUNITY OF THE PRESIDENT CANNA

The variety The President was symptomless as received from Washington, D. C., and from Brooklyn, N. Y., in each of which places it had been grown adjacent to mosaic cannas. No mosaic symptoms have been detected in this variety in the writers' scattered observations elsewhere. In parallel tests *Myzus persicae* transmitted canna mosaic to *Canna indica* from the varieties King Humbert (4/5), Richard Wallace (7/10), and Wyoming (5/5), but failed to transmit from The President (0/5). The President was then inoculated from Richard Wallace by this vector without effect (0/2) while *C. indica* in a parallel trial was infected (3/3). Transfers of *M. persicae* from these inoculated plants of The President to *C. indica* were without effect (0/8), but parallel transfers from mosaic *C. indica* to *C. indica* produced typical canna mosaic symptoms (3/3). These experimental results show that The President is not a symptomless carrier of canna mosiac, and offer strong evidence that this variety is immune from the disease.

DISCUSSION AND CONCLUSIONS

Canna mosaic as studied by the writers is considered the same as the mosaic of *Canna indica* previously reported from the Philippines. Transmission by *Aphis gossypii* and by *A. maidis* was confirmed. The principal

discrepancy is the failure of our virus to infect *Musa textilis*. *Canna glauca* is reported as an additional host species. Experimental transmissions by leaf rubbing and by additional vector species *Macrosiphum solanifolii, Myzus circumflexus,* and *M. persicae* were established. Evidence is presented that the popular red-flowered ornamental canna variety The President is immune from canna mosaic.

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INFECTION OF TOMATO FRUITS BY COLLETOTRICHUM PHOMOIDES[1]

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The fungus *Colletotrichum phomoides* (Sacc.) Chester has long been considered a wound pathogen on ripe tomatoes. Arthur (1), Chester (2, 3, 4), Halsted (9), Gueguen (8), and others were able to transmit the disease from one ripe tomato to another only by wounding the inoculated fruits. Davidson (5) concluded from field observations that natural infection must take place through microscopic cracks in the cuticle of the fruits. Doolittle (6, p. 27) stated that *C. phomoides* can infect apparently unwounded tomatoes. This statement was based on studies in which healthy tomatoes were sprayed with a suspension of spores of the anthracnose fungus.[3]

Tomato anthracnose develops ordinarily on ripe fruits but the failure to control the disease by application of fungicides to near-ripe or ripe fruits suggests that infection takes place some time prior to ripening (16, 17).

A study was made of some of the factors related to infection of tomato fruits by *Colletotrichum phomoides*, with emphasis on the mode of infection and the stages of maturity at which the fruits are susceptible to infection.

METHODS

Experiments were conducted on greenhouse-grown tomatoes using two red-fruited varieties, Early Baltimore and Garden State. In preliminary studies field-grown tomatoes proved unsatisfactory because of the high percentage of natural infection. The greenhouse plants were started in pots and after several weeks were transplanted to the bench, where they were supported by heavy twine and pruned to two branches. A date tag was attached to each blossom shortly after pollination when the ovaries became slightly swollen.

The inoculum used was obtained from five-day-old cultures of *Colletotrichum phomoides* on potato-dextrose agar slants. A small amount of water was added to each slant and a spore suspension prepared by gently scraping the surface with a wire loop. Spores were then washed[4] by cen-

trifuging through two changes of water. Several ml. of this suspension were poured onto pads of moist cotton approximately two inches in diameter and these were applied to the fruit surfaces. Detached fruits were placed in moist chambers for two days. Fruits inoculated on the vine were covered with waxed paper bags for two days to conserve moisture.

In histological studies of the mode of infection, fruits were inoculated by the methods just described or in some cases with spore suspensions applied in droplets to marked areas on the fruits. Inoculated areas were cut from the fruits at various time-intervals ranging from twenty-four hours to several weeks. The specimens were fixed in FAA, dehydrated in ethyl alcohol, cleared in xylol, and imbedded in paraffin according to the usual methods. Sections were cut 10 microns in thickness and stained with safranin and fast green. A few sections were stained with Flemming's triple stain after decolorizing in Stockwell's solution. Temporary mounts for observation of the spores and appressoria on the fruit surfaces were made by mounting pieces of tomato skin in lacto-phenol containing 0.1 per cent cotton blue.

FRUIT INFECTION

Wounding

In studying the relation of wounding to infection five shallow wounds were made with a sharp needle in the epidermis of each tomato at the time of inoculation. Fruits of various ages were inoculated and allowed to ripen fully on the vines. They were then picked and stored at 80° F. for two weeks. No attempt was made to control humidity. As the lesions appeared care was taken to determine whether the lesions developed from wounds or in the surrounding apparently unwounded areas. The percentages of fruits developing lesions from wounds and the percentage showing lesions in the unwounded areas are presented in table 1.

TABLE 1.—*Infection of wounded tomato fruits*

Variety	Age when inoculated	Number inoculated	Percentage developing lesions from wounds	Percentage developing lesions in unwounded areas
Early Baltimore	10 to 20 days	8	0	50
	20 to 40 days	5	60	80
	40 to 60 days	11	100	72
	Control, uninoculated	16	.. ..	0
Garden State	10 to 20 days	10	0	70
	20 to 40 days	8	25	87
	40 to 60 days	8	62	100
	Control, uninoculated	15	....	0

These data indicate that as the fruits approach maturity the pathogen can readily gain entrance through wounds. Young fruits, however, are not easily infected in this manner. Wounding of young fruits causes a corking of the surrounding tissues which effectively prevents further development of the fungus. Wounding, in the manner described, is not necessary to obtain infection. Inoculation of young fruits produces typical lesions only on apparently unwounded areas. Infection of mature fruits can also occur on unwounded areas.

Maturity

The relation of maturity of fruits to the susceptibility to infection by

TABLE 2.—*Infection of unwounded tomato fruits by Colletotrichum phomoides in relation to the age of the fruits when inoculated*

Variety	Age when inoculated	Number inoculated	Percentage of green fruits with stylar rot	Percentage of ripe fruits with typical lesions
Early Baltimore	5 days	8	25	0
	10 days	10	20	0
	15 days	13	7	33[a]
	20 days	14	0	57
	30 days	17	0	88
	40 days	25	0	80
	50 days plus, fruits green	21	0	85
	50 days plus, fruits pink to ripe	15	0	80
	Control, uninoculated	16	0	0
Garden State	5 days	4	75	0
	10 days	13	30	44[a]
	15 days	16	0	62
	20 days	23	0	65
	30 days	11	0	63
	40 days	14	0	85
	50 days plus, fruits green	14	0	92
	50 days plus, fruits pink to ripe	7	0	85
	Control, uninoculated	15	0	0

[a] Calculated on the basis of the fruits which reached maturity.

Colletotrichum phomoides was investigated by inoculating unwounded tomatoes at various ages, viz., 5, 10, 15, 20, 30, 40, 50 days, and later as the fruits colored and ripened. The fruits were picked and stored for two weeks in the same manner as previously described.

The percentages of infection (Table 2) indicate that the fungus can penetrate the cuticle and cause incipient infection at a very early stage in fruit

development.   There is some increase in susceptibility to this type of infec-
tion as the fruits mature.

A certain number of the fruits inoculated when 15 days old or less devel-
oped a stylar rot 20 to 30 days after inoculation.   The area around the style
became black to dark brown and black sclerotial bodies of the fungus devel-
oped in this dry, rotted region.   The rot was much more extensive in the
interior of the fruits than it was on the exterior.   Seeds, as well as the tissues
of the walls of the seed cavities, showed discoloration and rotting.   *Colleto-
trichum phomoides* was repeatedly isolated from the affected tissues.   Rot-
ting in this instance may have followed injury to the styles at the time of
inoculation.

Temperature Relation

Nightingale and Ramsey (13) reported that *Colletotrichum phomoides*
was unable to grow on culture media or in wounded tomatoes at temperatures
above 95° F.   The optimum temperature for growth of the fungus, com-
puted from 6 days' development, was shown to be 80° F.   The present work
shows that infection of apparently uninjured fruits is obtained over a similar
wide range.

Unwounded, ripe tomatoes were inoculated by the method previously
described and placed in moist chambers for two days.   During inoculation
and subsequent storage, fruits were kept at 50, 60, 70, 80, 90, and 100° F.
At 50° F. lesions developed slowly after approximately twenty days.   In-
fection was readily obtained at 60 and 70° F., lesions being produced in
five to eight days.   Most rapid development of lesions was at 80° F.   At
this temperature they often appeared within three days.   Lesions produced
at 80° F. and below were similar in appearance to those on field-infected
fruits.   A few lesions developed at 90° F. after approximately seven days.
These few lesions were very small, sunken, and dry.   No lesions developed at
100° F.

The time required for the appearance of lesions was not constant for any
one temperature.   The rapidity with which lesions developed was dependent
not only upon temperature but also upon maturity of the fruits at inocula-
tion.   Large green tomatoes could be infected in the manner just described
but the appearance of the lesions in this case was delayed until after the
fruits had ripened.

HISTOLOGICAL STUDIES ON PENETRATION AND INFECTION

Fungi of the genera *Colletotrichum* and *Gloeosporium* characteristically
enter the host plant by means of penetration tubes which develop below
appressoria.   Simmonds (15) studied in detail the penetration of fungi
causing the anthracnose diseases of banana, papaw, and mango.   In these
cases an extremely fine thread develops from a short peg-like projection
at the base of the appressorium.   The fine thread penetrates the cutin layer
and broadens in contact with the cellulose wall of an epidermal cell to form
a well-defined hyphal mass between the cutin layer and the cell wall.

Spores of *Colletotrichum phomoides* germinate readily in droplets of water either on tomato fruits or on glass slides. At varying intervals after germination the fungus forms appressoria on the hyphae. On tomato fruits the appressoria may form on the ends of very short hyphae protruding from germinating spores, or they may develop after extensive mycelial growth. Commonly more than one appressorium is formed from a single spore.

Studies of stained sections prepared from inoculated tomatoes show that a penetration tube grows from the lower side of the appressorium into the cuticle (Fig. 1, A, Fig. 2, C, D). This tube is constricted at the point of entry into the cuticle and is swollen midway through the cutin layer. A nucleus is often evident in the penetration tube soon after the tube grows into the cutin layer (Fig. 2, D). Upon contact with the host cell wall the tip of the penetration tube enlarges slightly, pushing the host cell wall inward and away from the cutin layer. These steps in the penetration of the cutin layer have been observed in tomato fruits varying in age from fifteen days to red-ripe maturity.
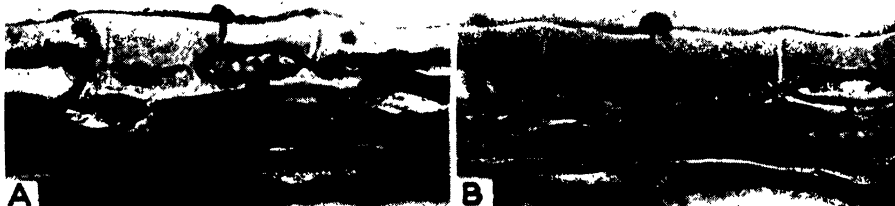
FIG. 1. Penetration beneath appressoria of the cuticle of a Garden State tomato 60 days old, 6 days after inoculation. A. Penetration tube has grown through the cutin layer and is in contact with the epidermal cell wall. B. Fungus hyphae developing from a penetration tube. The hyphae have pushed aside the cell wall and are growing between the cell wall and the cutin layer. The cell contents and the walls and contents of adjacent cells are strongly stained with safranin. Photographs approx. 408 ×.

In green fruits the growth of the pathogen is limited to the area between the cutin layer and cell wall. The cell wall in contact with the penetration tube is heavily stained by safranin. The contents of the affected cell often take the stain more strongly than the adjacent cells. A small swelling in the cell wall usually develops on the side opposite the fungus.

In ripe fruits the hyphae, which have penetrated the cuticle either recently or at some earlier stage when the fruits were green, continue enlargement between the cutin layer and the host cell wall (Fig. 2, E). Lateral development from one epidermal cell to another is apparently prevented by the cutin deposited between the cells. The wall in contact with the fungus is pushed aside by several swollen hyphae. The fungus slowly dissolves the cell wall and the contents are pushed into a small mass at the base which stains with safranin (Fig. 1, B). Adjacent cell walls in the hypodermis and epidermis also show this characteristic staining in advance of the fungus.

From the epidermal cell the fungus grows into the hypodermal layers but never laterally into the adjacent epidermal cells. The development is both inter- and intracellular. When it is intracellular the contents of the cell are pushed aside. The hyphae are swollen, with some constrictions, and limited in extent. At this stage there is a certain amount of lateral development in the hypodermis. The fungus continues to cause discoloration of cell walls and contents in advance of its development.
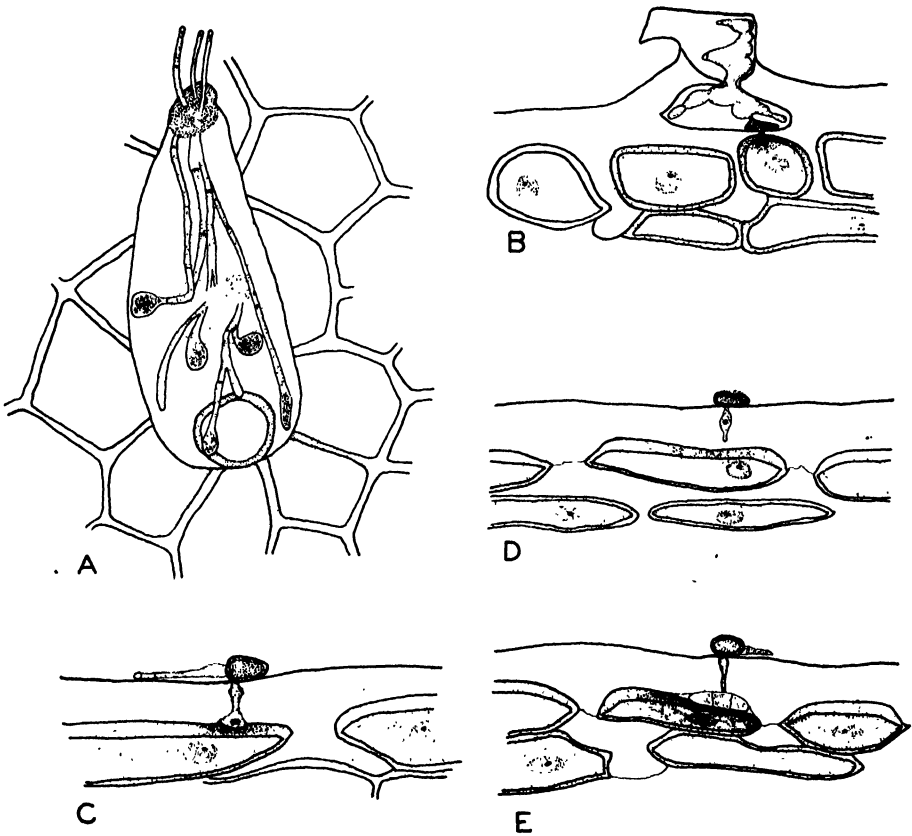
FIG. 2.   A. Garden State tomato 28 days old, 2 days after inoculation, showing invasion of broken epidermal hair by hyphae.   B. Early Baltimore tomato 33 days old, 4 days after inoculation, showing development of hyphae in epidermal hair and penetration beneath appressorium at base of hair.   C. Same tomato showing penetration tube growing through cutin and swelling in contact with cell wall.   D. Garden State tomato 60 days old, 6 days after inoculation, showing penetration tube in cutin.   E. Same tomato showing swollen hyphae developing between cutin and cell wall.   Drawn with aid of camera lucida, approx. 573 ×.

The hyphae change in form and manner of growth when they enter the parenchyma cells below the hypodermal layer. Growth is rapid and more extensive than that observed in the epidermis and hypodermis. The hyphae ramify in all directions showing both intra- and intercellular development. The cell walls and cell contents are penetrated readily and the fungus no longer follows the contours of the cell walls. The hyphae are much finer

than those in the hypodermal layer and are no longer constricted. The cell walls and cell contents are not discolored in response to the fungus. At this stage there is a partial collapse of the cells of the hypodermal layer and the affected parenchyma cells. As the lesion enlarges the fungus continues its development in the parenchyma cells and may also develop laterally in the hypodermal layer and in the cells of the epidermis.

It was noted in temporary mounts of the skin of inoculated fruits that the fungal hyphae were often associated with the epidermal hairs. The fungus penetrated and developed within the cells of some hairs (Fig. 2, A). The hyphae were generally tipped with appressoria near the base of the hair. On ripe fruits lesions occasionally appear to develop from the base of such affected hairs. This penetration and development seems to be essentially the same as that described for cuticular penetration. The fungus forms appressoria at the base of the hair from which it penetrates to adjacent cells of the hypodermal layer (Fig. 2, B). In green fruits the growth of the fungus is arrested at the first cell it contacts beneath the hair. It should be emphasized that the common method of entry of the pathogen is directly through the cuticle, although the epidermal hairs on occasion appear to be infection courts.

Various types of cracks in the cuticle of field-grown tomato fruits have been reported by Young (18) and Groth (7). Davidson (5) has suggested that these are the primary infection courts for *Colletotrichum phomoides*. In the course of the present studies cracks were rarely observed in the cuticle of greenhouse-grown tomatoes. Since the fungus appears to be admirably suited for direct penetration of the cuticle, it is unlikely that cuticular cracks are important in penetration and infection of the fruits.

RELATION OF pH CHANGES IN FRUIT TO GROWTH OF PATHOGEN

One of the striking features of tomato anthracnose is that lesions develop only on ripe or ripening fruits. Since the fungus can exist within or beneath the cutin layer from the time the fruit is 10 days old until maturity, it must be assumed either that there are factors present in green tomatoes which inhibit further development of the fungus or that some material necessary for fungal growth develops during the ripening process. It has been suggested that changes in pH may be a controlling factor. There is no evidence that this is the case.

Nightingale and Ramsey (13), working with tomato anthracnose, concluded that other changes in the fruit during ripening are more important than changes in acidity in influencing the development of decay. They found that the average pH of the green tomatoes which they studied was 4.7 and that of ripe tomatoes 6.01. Their conclusions were based on the growth of *Colletotrichum phomoides* on potato-dextrose agar adjusted to these two values.

Hamner and Maynard (10), reviewing the factors influencing the nutritive value of the tomato, reported that figures of various workers on the pH

of ripe tomatoes range from 3.8 to 4.61. Hoffman, Krause, and Washburn (11) stated that acidity increases steadily during the period of fruit enlargement, and most rapidly as the fruits approach full size, but decreases during the ripening process. They did not report the pH of the fruits at various stages of maturity.

In the present investigation, tomatoes of various ages were macerated individually in a Waring Blendor and portions of this material were used to determine the pH with a Beckman pH Meter. In some tests certain sections of individual fruits were blended with a mortar and pestle and the pH determined in a similar manner.

Tomatoes less than 25 days old have a pH between 4.7 and 5.05. During the subsequent development there is a slow decrease in pH which lasts 20 to 30 days depending on the variety. This is followed by a sharp decrease to a point between pH 3.9 and 4.2 just prior to the development of red color
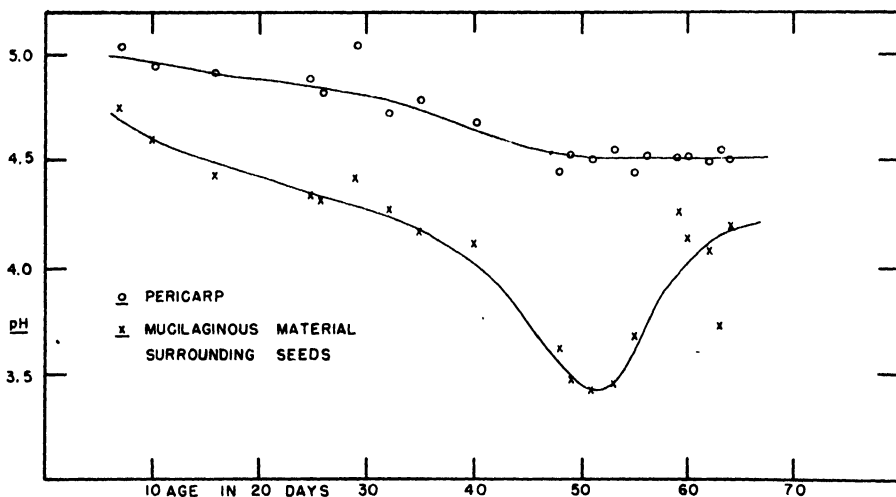
FIG. 3. The pH of the pericarp and the mucilaginous material surrounding seeds of Early Baltimore tomatoes of various ages.

in the fruits. The pH rises as the red color deepens so that mature tomatoes are between pH 4.2 and 4.6. There seemed to be slight consistent variations between the two varieties used but in the present work these differences were considered too minor to enter into the problem and could not be correlated with any differences in the amount of anthracnose.

Since the histological investigations demonstrated that the anthracnose fungus is confined mainly to the outer layers of the green fruits, the pH of various sections of Early Baltimore fruits was determined. The pericarp of the green and ripe fruits was found to have a consistently higher pH than the mucilaginous material around the seeds (Fig. 3). The pH of the pericarp drops slowly during the development and maturation of the fruit. On the other hand, the mucilaginous material surrounding the seeds shows a marked decrease in pH as the green fruits approach full size. During the

change from green to red-ripe the pH of this material rises and approaches that of the pericarp. This would indicate that the changes in pH noted in the whole fruits during maturation are the result of the changes in the interior of the fruits and not in the outer layers. It is in these outer layers that the anthracnose fungus remains apparently latent following penetration of the cutin layer. The inner walls of the carpels and the placental tissue have a pH between the two values presented in figure 3.

A series of cultural studies was run to determine the growth of *Colleto-trichum phomoides* on media with a wide pH range. The fungus was grown on potato-dextrose agar containing 2.5 gm. of K_2HPO_4 per liter. The pH was adjusted to various levels by aseptic addition of 50 per cent citric acid
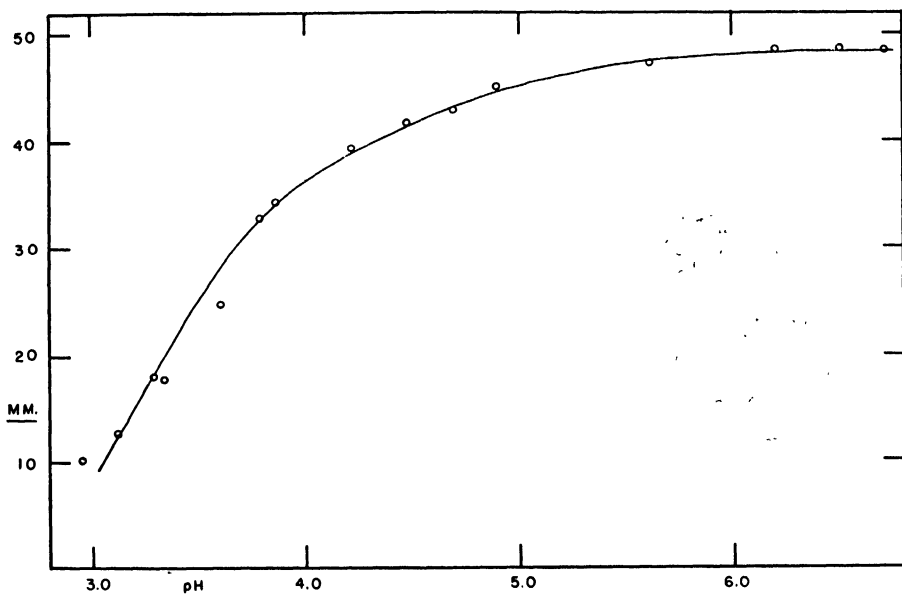
FIG. 4. The relation of pH to the growth of *Colletotrichum phomoides* on potato-dextrose agar. Diameter of colonies after five days at 80° F.

just before the agar was poured into Petri dishes. While the plates were being poured the pH of each medium was determined with a Beckman pH Meter. The agar was inoculated with a disc 1 mm. in diameter from the edge of a young colony of *C. phomoides*. The cultures were incubated at 80° F. for five days. The size of the colonies was determined by averaging the extreme diameters.

The pathogen grows well in culture within the extremes of pH found in tomato fruits (Fig. 4) and is not markedly inhibited unless the pH is lower than that found in green or ripe fruits. The range of pH found in the pericarp of tomatoes, pH 4.5 to 5.0, appears to be admirably suited to the growth of *Colletotrichum phomoides*.

It is apparent that the changes in pH which occur in the fruits during ripening do not influence directly the development of anthracnose on ripe

tomatoes. The inoculation studies demonstrated that *Colletotrichum phomoides* may occasionally penetrate at the style of the very young fruits and develop in the interior. In this stylar infection, the pericarp, and in particular the hypodermis and epidermis, are the last tissues to be invaded by the fungus. The inner tissues of the green tomatoes have a pH at which minimum development of the fungus would be expected. For this reason there must be factors in the pericarp other than pH which inhibit the growth of the fungus in green fruits.

Since the anthracnose fungus is confined to the epidermis and hypodermis during the latent stages of infection and no attempt was made to separate these tissues from the remainder of the pericarp, it is obvious that the pH of the immediate environment of the fungus has not been determined.

<div align="center">DISCUSSION</div>

The present studies show that the pathogen causing tomato anthracnose is capable of penetrating the cuticle of green as well as ripe tomatoes, although lesions are produced only on ripe fruits. In green fruits the fungus remains latent until ripening begins. The fungus is able to push between the cell wall and the cutin layer to a limited extent but there is no evidence that it is capable of dissolving or penetrating the wall while the fruit is still green. The growth of the fungus in green fruits may be restricted by the composition of the cell walls. These cell walls, in addition to cellulose, are composed of protopectin which is partially converted to pectin in the ripening process. Simmonds (15) suggested that the latent condition of the banana anthracnose fungus in green fruits may be caused by the inability of the fungus to attack the protopectin of the cell walls. During ripening the protopectin is converted to soluble pectin which the fungus is presumably able to attack. Le Crone and Haber (12) have shown that protopectin is converted to soluble pectin during ripening of tomato fruits.

Sando (14) discussed the process of ripening in the tomato and pointed out that the most striking change which occurs is that undergone by the carbohydrates. Sugars increase during the maturation of the fruit and starches decrease during the same period of time. These changes, however, can not be expected to affect drastically a fungus which exhibits very little selectivity in regard to the source of carbon in its nutrition.

The relative amount of moisture is probably the controlling factor influencing infection under field conditions since the fungus can infect the fruits over a wide range of temperatures. The fungus can continue development in the ripe fruits over a similar wide temperature range. Therefore, if the fruits are infected in the field, little can be done by controlling storage temperatures to prevent the development of lesions as the fruits mature. Treatment of the harvested fruits with fungicides prior to storage can be expected to give little appreciable control of anthracnose since the fungus is latent beneath the cutin layer.

Wounds occurring naturally or produced in handling can not be considered an important factor in the development of this disease. Cultural practices which reduce wounding or breeding work to minimize cuticular cracking can not be expected to produce reductions in the amount of anthracnose. The pathogen penetrates the uninjured cuticle with ease and it is likely that this type of infection is responsible for serious outbreaks of the disease.

Since the fungus is capable of remaining latent in green tomato fruits, effective control with fungicides is dependent upon good coverage of each fruit throughout the entire period of its development on the vine. Sprays and dusts applied at the time the fruits are ripening may give a slight reduction in the amount of anthracnose but such treatment can not be expected to affect the latent infection which may already be present in the fruits.

SUMMARY

A study was made of some of the factors related to infection of tomatoes by the anthracnose fungus, *Colletotrichum phomoides*, using greenhouse-grown fruits.

When the pathogen was introduced through wounds in fruits on the vine, infection was readily obtained on large green or mature fruits. Infection was obtained on unwounded areas of tomato fruits less than 20 days old but not through wounds on these fruits.

Unwounded tomatoes were susceptible to infection from the time the fruits were 10 days old until maturity. The susceptibility to infection increased with the age of the fruits. The fungus, in green fruits, remained latent until the fruits ripened and produced typical lesions at that time.

Detached, mature, unwounded tomato fruits were readily infected through a wide range of temperatures below 90° F. Lesions were produced most rapidly at 80° F.

Colletotrichum phomoides penetrates the cuticle of the fruit by means of a penetration tube which grows from the lower side of the appressorium. The infection tube in green fruits swells slightly on contact with the cell wall but remains latent between the cell wall and the cutin layer. As the fruits ripen the fungus enlarges and produces swollen, inter- and intracellular hyphae in the hypodermis. Upon contact with the parenchyma cells the fungus develops many fine hyphae which grow rapidly through the walls and cell contents. Microscopic cracks in the cuticle are not believed to be a factor in infection.

There is no apparent relation between the pH of the fruits during ripening and the development of lesions. The fungus grows well within the ranges of pH found in tomatoes.

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ANTHRACNOSE OF TOMATO[1]

J. B. KENDRICK, JR. AND J. C. WALKER

INTRODUCTION

The anthracnose fungus was described on tomato (*Lycopersicon esculentum* Mill.) by Saccardo (13) in 1878 as *Gleosporium phomoides*. It was first noted in the United States in 1891 by Chester (2), who, believing the fungus to be distinct from that of Saccardo, since setae were produced in the acervulus, designated it as *Colletotrichum lycopersici*. Further investigation, however, revealed that the organism varied widely with regard to production of setae, sometimes none being produced. After two years of study Chester concluded that the American organism was the same as that described by Saccardo and in accord with rules of nomenclature he named it *Colletotrichum phomoides* (Sacc.) Chester (3). Gueguen (6) observed the disease in France in 1892.

Although anthracnose has been of frequent occurrence on the tomato, particularly in the canning region from New Jersey to Missouri, little research has been reported on the life history of the causal organism or upon the disease cycle. The purpose of this investigation was to study the method of overwintering of the fungus, the source of primary inoculum, the disease cycle on foliage and fruits, the host range of the fungus and the influence of various factors upon the development of the organism in pure culture.

THE CAUSAL ORGANISM

Source and Variability of Cultures

Isolates from 147 naturally infected tomato fruits from Racine and Kenosha, Wisconsin, showed a considerable range of growth characters on potato-dextrose agar.[2] All fell within the characteristics of acervulus and spores described by Chester (3). Some 14 fairly distinct types of cultural characteristics were recognized. In most of these, spores were not produced abundantly enough to accumulate in conspicuous spore masses but all produced some spores. They appeared to be chiefly of the MC type described by Tompkins and Hansen (15) for *Gleosporium thumenii* (Sacc.) var. *tulipae* Tompkins and Hansen. Isolates also varied in the color of the substrate; in prostrate or aerial growth of mycelium; and in the number, size, and arrangement of the stromata. In general the variability appeared to be of the same kind as that reported for *Colletotrichum destructivum* O'Gara by Chilton (4), for *C. capsici* Syd. by Ramakrishnan (11), and for *G. thumenii* var. *tulipae* by Tompkins and Hansen (15).

Three isolates were selected for use in this study. Their growth on potato-dextrose agar is characterized as follows:

[1] The writers are indebted to Eugene Herrling for preparing the illustrations, and to John H. Owen for assistance in some of the field work.

[2] 200 gm. potato; 20 gm. dextrose; 17 gm. agar; 1000 ml. distilled water.

Cp1 (Fig. 1), isolated from tomato, produced aerial mycelium and relatively large stromata scattered uniformly over the surface of the agar. Spores were produced sparsely and were similar in size and shape to those of Cp5. It was similar to the MC type of Tompkins and Hansen (15).

Cp4 (Fig. 1) was isolated from naturally infected tomato by W. J. Hooker. This isolate was distinct from all other strains in that the stromata were white in contrast with the black stromata of other isolates. The white mycelium was submerged; sporulation was profuse and pink masses of spores were conspicuous on the surface of the culture. The spores were straight with subacute ends, continuous, and uninucleate and they each contained two or three vacuoles. Measurements of 100 spores from each of five
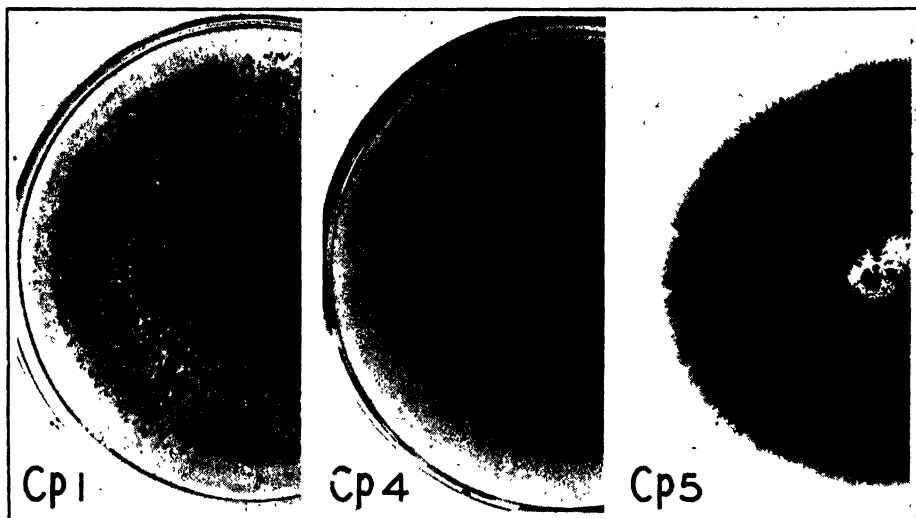
FIG. 1. Appearance of isolates Cp1, Cp4, and Cp5 grown on potato-dextrose agar at 24° C.

single-spore sub-isolates showed a range of 13.8 to 23 × 3 to 4.6 μ, with an average of 18.0 × 3.9 μ. Setae were sparse and stiff; they were always hyaline as compared with the black setae of all other isolates studied. No cultural variation was noted in five successive transfers of 50 single spores for each transfer. Spore production and cultural characters remained constant through successive mass transfers during five years. Albino strains were reported by Caldis and Coons (1) in single-spore cultures of *Physalospora malorum* (Berk.) *Shear* (imperfect stage), *Colletotrichum lindemuthianum* (S. & M.) Bri. & Cav., *Cladosporium fulvum* Cke., and *Septoria apii* (B. & C.) Chester. The variants of *P. malorum* and *S. apii* soon reverted to the parent type; the other two variants retained the albino character for two years. They all sporulated sparsely, they arose as cultural variants and eventually reverted to the parent type; Cp4 was very stable, it was secured as an isolate from naturally infected tomato, and it sporulated abundantly.

Cp5 (Fig. 1) was derived by five successive single-spore transfers from
a sector of a strain isolated by W. J. Hooker from naturally infected tomato.
Sporulation was practically doubled in amount with each successive transfer.
The profusely sporulating strain was, therefore, of the C type as described
by Tompkins and Hansen (15). A slight amount of aerial mycelium was
formed, and relatively small black sclerotia were produced in such abundance
as to aggregate in a crusty layer on the surface of the agar. The spores
were uninucleate and were similar in shape and vacuolation to those of
Cp4. Measurement of 100 spores from each of five single-spore sub-isolates
showed a range of 15 to 25 × 3 to 5 μ with an average of 19.4 × 4.4 μ.

<h3 style="text-align:center">RELATION OF TEMPERATURE TO GROWTH</h3>

Isolates Cp1, Cp4, and Cp5 were used. Petri dishes of uniform size
containing 15 ml. each of potato-dextrose agar were each inoculated at the
center with a 3-mm. disc cut from the periphery of an actively growing
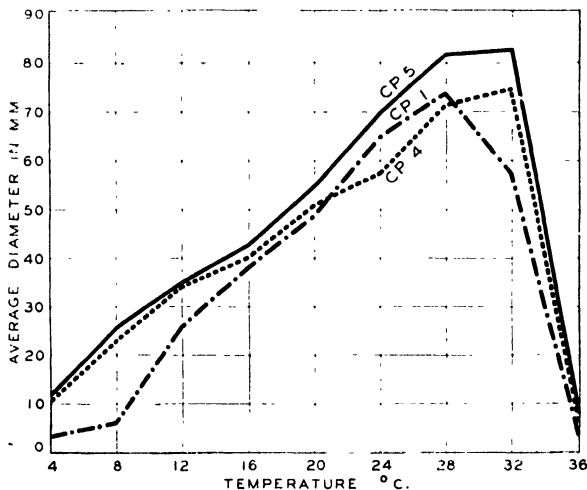
FIG. 2. Relative growth of three isolates after nine days at various temperatures.

culture. Three dishes of each isolate were placed in incubators at 4°, 8°,
12°, 16°, 20°, 24°, 28°, 32°, and 36° C. The plates were wrapped in wax
paper to maintain equal moisture conditions. Daily measurements were
made of two diameters per colony taken at right angles to each other and
averaged. Three experiments gave similar results. The results at nine
days from one experiment are reported graphically in figure 2. The MC
isolate (Cp1) had a slightly lower optimum than the sporulating isolates.
Staling effects were evident with Cp1 at 20° and 24°. When cultures show-
ing no growth at 4° and at 36° were transferred to 24° the normal growth
rate for the latter temperature resumed. Sectors varying in size and ar-
rangement of stromata and in sporulation appeared at temperatures from
20° to 32° in Cp1 and Cp5. In all strains stromata appeared when the
diameter of the colony reached about 20 mm. Usually spores were formed
two to three days after the formation of the stromata.

Relation of Temperature to Spore Germination

Spores from one-week-old cultures of Cp4 and Cp5 were suspended in distilled water and adjusted in concentration to 100 to 150 spores per low power microscopic field ($\times$ 350). Drops were placed on water agar plates. One hundred spores from each of three plates per temperature were counted. Spores of Cp4 did not germinate at 4° and 36° C. up to eight days; those of Cp5 all germinated at 4° in 8 days and at 36° in two days. Complete or nearly complete germination of spores was reached most promptly with Cp4 and most promptly with both at 20° to 32°. In both isolates spores usually germinated by a single germ tube forming at one end at 4° to 28° and by tubes from both ends at 32° and 36°.

Relation of Carbon Source

A modification of Duggar's solution[3] was used as the basic nutrient in physiologically clean 125-ml. Erlenmeyer flasks to which weighed filter paper cones were added as a substrate for fungus growth. The carbon sources were: glucose, d-levulose, lactose, d-xylose, dextrin, inulin, sucrose, soluble starch, galactose, glycerine, and ethyl alcohol. Twenty-five ml. of a 10 per cent solution of the given carbon source was made up to 50.5 ml. with the basic nutrient. The initial reaction was approximately 4.5; the final reaction was 6 to 7. Each flask was inoculated with 0.15 ml. of a suspension of spores of strain Cp5. In another series 1.7 gm. of agar per 100 ml. was added to the solution and three Petri dishes per treatment poured with 20 ml. of the medium per dish. The plates were inoculated with a 3-mm. loop of spore suspension. All cultures were incubated at 22° C. Three replicates were made for each treatment. Growth in the liquid cultures was determined after 21 days by filtering off the fungus mat and filter paper, drying to a constant weight at 110° C. and subtracting the weight of the filter paper from the total weight. Averages of replicates are presented. Spore production on the agar cultures was estimated by the appearance of spore masses, when visible, or by the amount of spores obtained in drawing a water-filled loop across the surface once. Production of setae was estimated by examination of the culture with a binocular. The results are presented in table 1.

Glucose, dextrin, and soluble starch were good carbon sources in liquid and solid media. Lactose and inulin were better sources on solid than in liquid medium. The poorest sources of carbon were d-xylose and ethyl alcohol. Best sporulation was with inulin, glucose, sucrose, dextrin, and lactose and poorest with d-levulose and soluble starch. No spores occurred in d-xylose, ethyl alcohol, and the control. Formation of setae was usually correlated with spore production, the exception being ethyl alcohol with which there were many setae but no spores. No setae were formed with d-xylose and in the control.

[3] 10.0 ml. 0.5 M KNO_3; 10 ml. 0.25 M KH_2PO_4; 5.0 ml. 0.10 M $MgSO_4$; 0.5 ml. 0.001M $FeCl_3$; 0.005 ml. of a minor element solution consisting of 2.818 gm. H_3BO_3, 0.04 gm. $CuCl_2\cdot 2H_2O$, 0.03 gm. $ZnCl_2$, and 0.39 gm. $MnCl_2$ per liter; made up to 1000 ml. with distilled water.

TABLE 1.—*Relation of carbon source to growth and sporulation of Colletotrichum phomoides*

Carbon source	Weight of mat in liquid culture	Diameter of colony on agar at 5 days	Relative order as to	
			Sporulation	Formation of setae
	Gm.	*Mm.*		
None	0.00	0.0	None	None
Glucose	0.96	50.0	2	1
D-levulose	0.80	31.0	5	3
Lactose	0.72	40.3	4	2
D-xylose	0.26	0.0	None	None
Dextrin	0.87	50.0	4	4
Inulin	0.67	44.3	1	1
Sucrose	0.82	39.0	3	3
Soluble starch	0.90	32.0	6	5
Galactose	0.00	....	..	
Glycerine	0.81	.		
Ethyl alcohol	0.13	18.0	None	2

Sporulation on Standard Media

Cp1, Cp4, and Cp5 were compared on media listed in table 2. The best sporulation with all three strains occurred in nutrient dextrose which might well have been due to accessory substances in the peptone and beef extract. Cp1, which sporulated poorly on potato-dextrose agar, produced spores profusely on this medium. By contrast, all 3 strains sporulated poorly in Steinberg's dibasic medium although setae were abundant in Cp4 and Cp5. Elliott's and Richard's media, which differ from Steinberg's in containing monobasic phosphate and a different source of nitrogen, supported good sporulation in Cp4 and Cp5 but not in Cp1. In Leonian's and Czapek's media spore production was moderate in Cp4 and Cp5 and sparse in Cp1.

Relation of Nutrition to Spore Germination

Spores from 14-day cultures of Cp4 and Cp5 were suspended in a 1 per

TABLE 2.—*Comparison of agar media used in study of spore and seta production*

Medium	Grams per 500 ml. of water of				
	Dextrose	Sucrose	$MgSO_4 \cdot 7H_2O$	KH_2HPO_4	Other
Steinberg's Dibasic	.....	25.0	0.3	0.24	1.0 NH_4OH 0.01 $FeCl_3$
Elliott	25.0	..	0.25	0.68	0.53 Na_2CO_3 0.50 asparagin
Nutrient Dextrose	5.0		....	. ..	1.5 beef extract 5.0 peptone
Leonian	6.13	...	0.60	0.60	3.13 malt extract
Richards	.. ...	25.0	1.25	2.5	5.0 KNO_3 0.01 $FeCl_3$
Czapek	....	.. ....	0.25	0.5	0.005 $Fe_2(SO_4)_2$ 1.0 $NaNO_3$ 0.25 KCl

cent dextrose solution, 1 per cent peptone solution, saline solution (0.85 per cent sodium chloride), and distilled water at concentrations of about 200 spores per low power field. Drops of a given suspension were placed on clean glass slides within Petri dishes containing 5 ml. of the medium absorbed by filter paper. During incubation at 28° C., 100 spores per drop were counted at frequent intervals. The averages of six replicates are given in table 3. Spores of Cp5 germinated more rapidly than those of Cp4. Most rapid germination occurred in peptone, next in dextrose. Cp5 germinated nearly as rapidly in distilled water as in dextrose, but Cp4 was retarded markedly. Saline solution was decidedly unfavorable.

TABLE 3.—*Influence of nutrient on germination of conidia of Colletotrichum phomoides*

Medium	Strain	Percentage germination at hours indicated								
		3	5	6	8	10	12	22	27	46
Dextrose	Cp4	0	0.0	0.0	0.0	0.0	0.0	4.3	27.1	96.1
	Cp5	0	0.4	0.9	1.0	1.3	1.5	23.3	63.2	90.0
Peptone	Cp4	0	2.0	3.5	6.2	6.5	7.6	28.2	90.0	90.0
	Cp5	0	2.5	4.5	13.5	23.0	37.5	90.0	90.0	90.0
Saline	Cp4	0	0.0	0.4	0.5	0.5	0.5	0.5	0.7	31.0
	Cp5	0	0.0	0.0	0.5	0.8	0.8	1.0	1.5	19.0
Distilled water	Cp4	0	0.0	0.0	0.4	0.4	0.4	0.5	9.1	43.0
	Cp5	0	0.0	0.0	0.8	0.8	1.2	29.0	73.5	90.0

Relation of Reaction of Medium to Growth and Sporulation

Potato-dextrose agar was adjusted to a range of pH values from 2.9 to 9.9. Plates were poured and inoculated with 3-mm. discs from cultures of Cp4 and Cp5 and incubated at 24° C. Growth of both strains was retarded decidedly at pH 2.9, there was moderate growth at 3.9, and good growth at initial pH values from 4.9 to 9.9. No sporulation occurred at 2.9; most abundant sporulation occurred at 3.9; sporulation was slightly less at 4.9 to 6.9; it decreased rapidly above 6.9 and was rare at 9.9.

Relation of Host Substrate to Growth, Sporulation, and Survival

Since concurrent studies showed that *Colletotrichum phomoides* is for the most part a saprophyte and pathogenic chiefly in connection with fruit decay, the relation of host substrate to growth and survival in the soil was studied. In previous sections it was shown, moreover, that the carbon and nitrogen sources and reaction of the substrate had a bearing on the extent of growth and sporulation. Green and ripe fruits, and green and dead stems were cut into small pieces and sterilized in pint bottles alone and in various combinations with soil and sand. They were inoculated with Cp4 and Cp5, incubated at 22° C. for 10 days, and observed. After one month they were set out of doors in November at Madison and left until May. During the winter period temperatures were below freezing for most of the time in December, January, and February.

Soil and sand alone were very poor media for growth. Best growth and sporulation occurred on green and ripe fruit tissue and green stem tissue alone and when mixed with sand. Poorest development occurred on these substrates in soil. Development on dead stems was intermediate whether alone or mixed with sand or with soil.

When the cultures were examined and attempts made to recover the fungus after overwintering, quite the opposite results were secured. The fungus was recovered from each substrate where soil was incorporated, but consistently with sand only when dead stems were used. When the various host tissues were used alone, the fungus was recovered only from dead stems inoculated with Cp5. The slow initial growth of the fungus in substrate mixed with soil appeared to be the best environment wherein the fungus was conditioned most favorably for winter survival.

<center>THE DISEASE CYCLE</center>

<center>*Overwintering of the Causal Organism*</center>

In the autumn of 1943 naturally infected tomato stems were collected from the field. They bore black, relatively inconspicuous stromata less than 0.5 mm. in diameter scattered irregularly over the surface (Fig. 3). *Colletotrichum phomoides* was isolated in each of 100 fragments tested. A crop of tomatoes was grown on the same field in 1944 and in 1945 with considerable damage from anthracnose. In the spring of 1946 fragments of tomato stems were collected; oftentimes they were partially buried by soil. Of 125 fragments tested, 111 yielded *C. phomoides*. When the stems were washed and placed in a moist chamber, setae and spore masses developed readily on the stromata.

An experiment was performed in this field in 1946 to determine whether the overwintering fungus or inoculum from an outside source was the more important in producing fruit infections later in the season. One thousand tomato plants were set out. Twenty-five plants were inoculated at the blossom stage by atomizing a spore suspension of the non-pigmented strain (Cp4) on the foliage at dusk. Twenty-five plants were inoculated in the same manner at the green-fruit stage. The non-pigmented strain was used to serve as a marker since it had not been obtained in the Madison area and all isolates from overwintered debris were darkly pigmented. Anthracnose developed after late summer rains in the middle of September. About 10 per cent of the fruits were infected throughout the field and the percentage was not higher on the inoculated tomato plants than on the uninoculated ones. Three hundred random isolations, 100 at each collection, were made from infected fruit in inoculated as well as uninoculated rows at two five-day intervals at the peak of the development of the disease. A pigmented isolate was obtained in every case. It was evident, therefore, that inoculum had come from overwintering debris and had not spread from artificially inoculated plants. This experiment was repeated in 1947 with identical results.

In a second experiment in 1947 a row of tomato plants with maturing fruit was inoculated with the non-pigmented strain through incisions in stem and ripe fruit. The plants were then covered with burlap for 40 hours. The non-pigmented strain was not recovered from inoculated stems and only from fruits which had been inoculated. Again all natural infection was from overwintering inoculum.

FIG. 3. Stromata of *Colletotrichum phomoides* scattered among fruiting structures of other fungi on dead tomato stems collected in a naturally infested field late in the autumn.

Seed Infection

Sterilized seeds were soaked for 24 hours in spore suspensions of Cp1, Cp4, and Cp5 and sown in sterilized soil. No infection occurred with Cp4 and only rarely did plants show infection with Cp1 and Cp5. Affected plants had small black lesions on the cotyledon, often extending down the cotyledon; or one or both cotyledons showed various degrees of collapse and chlorosis. The presence of the respective strain was confirmed by isolation. From naturally infected fruits 500 seeds were plated without further treatment; seven yielded the anthracnose organism. When 950 seeds from the same fruits were treated with 1 per cent sodium hypochlorite for 10 minutes and plated, none yielded the organism. When 1400 seeds from naturally infected fruits were planted in non-infested soil one plant was found to become infected. It appears, therefore, that the anthracnose organism may be transmitted with seeds from infected fruits.

Foliage Infection

Plants were placed in a moist chamber for two days, inoculated with Cp4 and Cp5, respectively, and kept in the moist chamber for two days

longer. Small brown necrotic spots bordered by chlorotic zones appeared
on the cotyledons, which turned yellow, shrivelled, and dropped prema-
turely. On leaflets, small necrotic spots surrounded by irregular chlorotic
areas appeared near the margins. Similar lesions without chlorosis ap-
peared on the stems. In the ordinary greenhouse atmosphere no sporulation
occurred on the lesions, but when plants were placed in the moist chamber
for four days setae and spores formed. Lesions on a leaflet are shown in
figure 4.

FIG. 4. A. Uninoculated tomato leaflet; B. Leaflet from plant inoculated with *Col-
letotrichum phomoides*. Small necrotic spots from near the margin surrounded by irregu-
lar chlorotic areas.

Five-week-old Bonny Best tomato plants were placed in a moist chamber
for 24 hours, inoculated, and returned to the moist chamber. Twenty-four
plants were removed at 18, 24, 48, 72, and 96 hours, respectively. At each
interval six plants were placed in greenhouses at 16°, 20°, 24°, and 28° C.,
respectively. The duration of the post-inoculation incubation in the moist
chamber was important. Only slight development occurred up to 24 hours.
The number and size of lesions were greater at 48 and 72 hours and still
greater at 96 hours' exposure. The lower leaves and the cotyledons showed

the greatest effect. The enlargement of lesions was checked upon removal of the plants from the moist chamber and the temperature of the greenhouse after removal of plants from the moist chamber had little or no effect upon disease development. Control plants sprayed with distilled water showed no signs of disease. Plants inoculated and placed in the respective greenhouses without moist-chamber treatment remained free from symptoms. In plants inoculated at four to nine weeks after transplanting there was a slightly greater amount of infection on the oldest plants inasmuch as they held more old leaves upon which most of the lesions occurred. Leaves containing lesions were incubated in moist chambers for three days at temperatures from 12° to 32° C. Setae and spores formed most abundantly at 28° and 32°, nearly as abundantly at 24°, and sparsely at 20°, 16°, and 12°. When plants were inoculated and placed in moist chambers at 28° and 16°, respectively, lesions appeared in three days at the high temperature and in 11 days at the low temperature.

Root Infection

White quartz sand was infested with *Colletotrichum phomoides* by incorporation of a pulverized cornmeal-sand mixture which had been sterilized and inoculated with the organism 17 days previously. Tomato seedlings were transplanted to the sand in crocks and watered with Hoagland's nutrient solution. A sand temperature of 28° C. and an air temperature of 24° were maintained for six weeks, when plants were removed and examined. Most plants had small inconspicuous necrotic flecks on the tap root and hypocotyl. Some of the lateral roots were rotted off. In general the injury, even under conditions favorable to the organism, had little retarding effect upon plant growth.

Fruit Infection

Forty green and 40 ripe fruits were inoculated with strains Cp4 and Cp5. Six inoculations were made on each fruit, three by placing a drop of the spore suspension on the uninjured surface, and three by making a puncture wound with a sterile needle through a drop of the spore suspension. The fruits were placed in a moist chamber for 48 hours at about 22° C. The results of the experiment are given in table 4. In no case did infection result from inoculation of the uninjured surface in either green or ripe fruits. However, a high percentage of infection by both strains resulted in both the green and ripe fruits when inoculations were made through wounds. Typical anthracnose lesions were produced by both strains on ripe fruits. Green fruits were slightly less susceptible to infection, and expansion of the lesion, consisting of dark necrotic tissue bordering the wound, was much less than in the ripe fruits.

Penetration of the ripe and green fruits inoculated with strain Cp5 was studied. On uninjured surfaces of both ripe and green fruits, spores germinated well at 24 hours and germ tubes measured 20 to 40 μ in length.

Appressoria formed at the ends of about three-fourths of the germ tubes, but no penetration hyphae were evident. No further development was seen even after 70 hours. On the injured surfaces of the ripe and green fruits, spore germination occurred abundantly after 24 hours, with germ tubes growing towards and into the wound. Appressoria formed on about 50 per cent of the tubes. A few parenchyma cells bordering the wound on green fruits were invaded at this time. At the end of 46 hours the wound had a sunken appearance. The fungus was more advanced in the parenchyma of ripe fruit than green fruit and after 70 hours the lesions on the ripe fruits were 2 to 3 cm. in diameter, compared to 3 or 4 mm. on green fruits. Penetration from cell to cell of the hypodermis of green and ripe fruits was studied in tissues cleared in a solution containing equal parts of acetic acid and ethyl alcohol, and stained with cotton blue in lactophenol.

TABLE 4.—*Infection of wounded and unwounded green and ripe fruits by two strains of Colletotrichum phomoides*

Strain	Ripe Fruits				Green Fruits			
	Wounded		Unwounded		Wounded		Unwounded	
	Inocu-lated	In-fected	Inocu-lated	In-fected	Inocu-lated	In-fected	Inocu-lated	In-fected
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Cp4	120	117	120	0	120	97	120	0
Cp5	120	116	120	0	120	101	120	0

The hyphae branched in all directions and penetrated the cell walls readily. The protoplasm of uninvaded parenchyma cells appeared to coagulate in advance of the pathogen.

Related Hosts

Six-week old tomato, eggplant (*Solanum melongena* L. var. *esculentum* Nees.) and pepper (*Capsicum annuum* L.) plants were placed in covered chambers and inoculated by atomizing with a spore suspension of Cp5. Five plants were used for each treatment, one plant serving as a control. The plants were incubated in a moist chamber at approximately 28° C. At six days, tomato plants inoculated with strain Cp5 had many pin-point necrotic spots on the cotyledons, which were chlorotic and curled. Primary leaves were infected predominantly at the margins, showing necrotic tissue surrounded by chlorotic areas. Eggplant cotyledons, which dropped prematurely, were yellowed and had numerous brown necrotic spots. Many dark green necrotic areas occurred on the inoculated leaves, and although small and unexpanded the area of effective photosynthesis was greatly reduced because of their abundance. The leaves yellowed and dropped prematurely. A typical diseased plant is shown in figure 5. The cotyledons of the young pepper plants did not become yellow or drop prematurely, but had numerous pin-point necrotic lesions consisting of collapsed tissue. The leaves had

tiny, brown, necrotic areas at the points where water droplets had persisted. Chlorosis did not occur on the pepper leaf to the extent that it had with tomato and eggplant leaves.   Symptoms on pepper are shown in figure 6. Control plants of the three hosts showed no signs of disease.   The organism was also isolated from naturally infected pepper fruits grown in a tomato field near Madison in which the disease occurred on tomato.

FIG. 5.   Left, uninoculated eggplant; right, eggplant inoculated with *Colletotrichum phomoides*.   Necrotic spots, chlorosis, and premature dropping occur on cotyledons and leaves.

DISCUSSION AND SUMMARY

The tomato anthracnose organism is important as a pathogen only on ripening tomato fruit, but under favorable conditions it will infect the foliage, stem, and root on which it causes very inconspicuous symptoms and no measurable damage.   The fungus grows well as a saprophyte on common media and can use a number of simple and complex carbon compounds as a source of energy.   It tolerates a wide range of hydroxyl- and hydrogen-ion concentration in the medium and sporulation occurs over a slightly narrower range.   A relatively high temperature (24° to 28° C.) is most favorable for sporulation, germination of spores, growth, and infection.   In spite of a rather wide range of adaptability to conditions in pure culture, the fungus dies out rapidly in soil.   It is probably not a good soil inhabitant but it survives winter conditions best as stromata on infected plant debris in intimate association with the soil.   How long it may remain as a soil invader in association with plant debris has not been determined, but it has been shown to infect fruits of pepper, grape, apple, eggplant, canna, and mango (7, 9),

and has been found on wheat and oats in association with root rot (14). While the fungus may be transmitted with seed and possibly in association with small necrotic lesions on transplants, the chief means of overwintering appears to be as stromata on plant debris. The organism is very mildly pathogenic on plant parts other than the ripening fruits. This is in accord with the report of Younkin and Dimock (17) and contrary to Rolfs (12) who
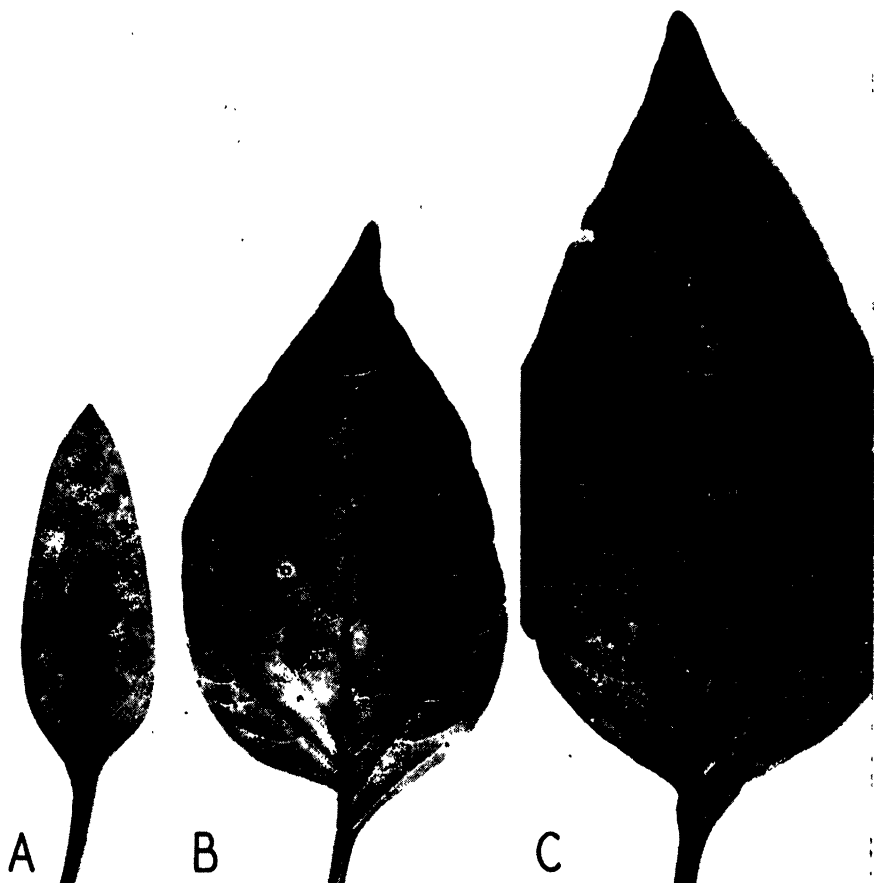
Fig. 6. Symptoms produced on pepper inoculated with *Colletotrichum phomoides*. A, cotyledon; B, young leaf; C, mature leaf. Chlorotic haloes surround lesions on the cotyledon and young leaf, while necrotic flecks occur on the old leaf.

reported earlier that the fungus caused extensive damage to the foliage. Infection of leaves, stems, and roots appears to take place without aid of wounds but injury of fruits is necessary before invasion occurs. It is assumed that wind-blown particles of soil are the chief cause of microscopic wounds on the fruits. When a marker (non-pigmented) strain was inoculated on the foliage and green fruits of plants exposed to natural infection from overwintering debris, all of the fruit infection appeared to come from

the latter source. This indicates that foliage infection is ordinarily a minor source of secondary inoculum. It would also indicate that crop rotation is the primary measure of control to be applied and that the success of chemical prophylaxis will depend upon how well the fungicide adheres to the fruit and protects it from spattering rain bearing soil particles and inoculum.

The mildly pathogenic property of the fungus, except upon maturing storage organs, is parallel with relatively similar situations found in some other anthracnose diseases, particularly those of pea (10), citrus (5), onion (16), and cucurbits (8).

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PHYTOPATHOLOGICAL NOTE

Botryosphaeria ribis, Perfect Stage of the Macrophoma Causing Ripe Rot of Muscadine Grapes.[1]—In 1941 Jenkins[2] reported a ripe rot of muscadine grapes caused by an undetermined species of *Macrophoma*. Further observation of the disease has resulted in the discovery of the perfect stage of this fungus, which is here tentatively referred to *Botryosphaeria ribis* Grossenbacher and Duggar. It might equally as well be placed in *B. berengeriana* DeNot. or other species of *Botryosphaeria*. *Botryosphaeria* is obviously in need of intensive study and revision. At present it is impos-
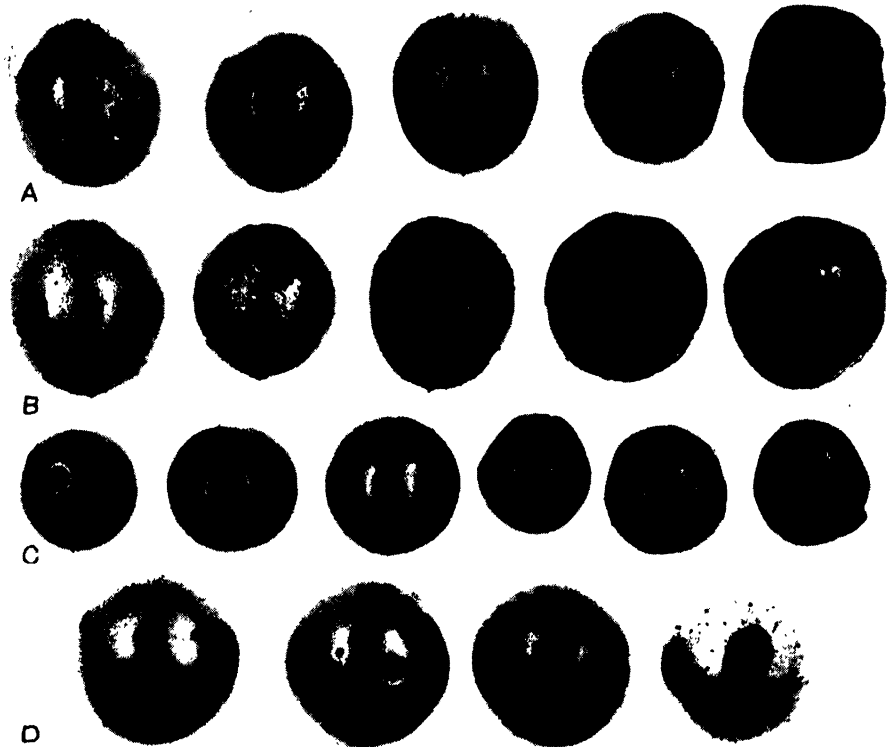
FIG. 1. Ripe rot of muscadine grapes. A. Howard. B. Lucida. C. Thomas. D. Willard. A, B, & C—×1; D—×1.2.

sible to determine species limits and the proper application of specific names in the genus.

The ripe rot caused by *Botryosphaeria ribis* appears when the berries reach full size, and it becomes more abundant as they ripen. The first symptoms of the disease are circular, flat or slightly sunken "bird's eye" spots one to four mm. in diameter (Fig. 1). The lesions are dark brown with small, tan or buff-colored centers in which a few *Macrophoma* pycnidia

[1] Paper No. 158, Journal Series, Georgia Experiment Station.
[2] Jenkins, W. A. Diseases of muscadine grapes. In Savage, E. F., et al. Further studies with the muscadine grape. Georgia Agr. Exp. Sta. Bul. 217. 1941.

are embedded. Usually this is the most severe expression of the disease. On the most susceptible varieties, however, a brown soft rot may spread over the berry from these lesions. Ultimately the entire berry may be rotted (Fig. 1). In later stages of the rot *Melanconium fuligineum* (Scrib. & Viala) Cav. often enters the berries and may be responsible for some of the damage. Berries in which the rot spreads drop from the vines. They shrivel slightly but do not form mummies. They are finally reduced to dry, hollow shells with pycnidia abundant over the entire surface.

Upon the basis of present evidence this disease appears to be of minor importance. Many muscadine varieties are almost immune. The disease is most severe on Thomas and, to a lesser degree, on Lucida, Howard, and Dulcet. Hunt, the chief commercial variety in Georgia, along with Creek, Scuppernong, Irene, and Yuga, is highly resistant. For example, in 1946 counts of the berries that dropped from two vines of each variety from the first of August to harvest time showed that on Hunt the disease was present on only 0.39 per cent of the fallen berries and seemed responsible for only 0.06 per cent of the drop. On Thomas it was present on 25.45 per cent of the fallen berries and seemed responsible for 11.31 per cent of the drop. Only 12.38 per cent of the total crop dropped from these vines, however, and it was estimated that even on Thomas only a 1.40 per cent crop loss could be attributed to berry drop resulting from infection with *Botryosphaeria ribis*. Control measures are considered unnecessary. *B. ribis*, along with *Melanconium fuligineum, Alternaria* sp., *Glomerella* sp., *Diaporthe* sp., and *Pestalozzia* sp. has been isolated also from blighted peduncles of flower clusters and berry clusters. On the peduncles it may be a secondary organism. Nevertheless, this aspect of the disease must be investigated further before a final estimate of its importance can be made.

Only the *Macrophoma* pycnidial stage of the fungus has been found on diseased berries. The pycnidia were spherical and measured 153–197 μ in diameter. The pycnospores were hyaline, unicellular, narrowly elliptical to ovoid, and rounded at either end. They measured $14.0–25.2 \times 5.6–8.4\,\mu$ (Av. 25 = $19.6 \times 6.6\,\mu$). Pycnidia filled with viable pycnospores have been found in the spring on berries that had dropped from the vines during the preceding season. The fungus, therefore, is capable of surviving the winter in the pycnidial stage. Pycnospores from an over-wintered berry measured $16.8–24.6 \times 5.6–7.8\,\mu$ (Av. 25 = $20.9 \times 6.4\,\mu$). Isolates of the fungus from rotted berries rarely fruited on agar media. Most of them, however, were induced to fruit on sterile grape stems partially immersed in water in culture tubes. Pycnospores formed under these conditions were elliptical to fusiform. Variations among measurements of pycnospores produced in culture by several isolates were as follows: $16.8–21.0 \times 5.0–7.0\,\mu$ (Av. 25 = $19.0 \times 5.8\,\mu$); $18.2–29.4 \times 5.0–5.9\,\mu$ (Av. 25 = $22.1 \times 5.5\,\mu$); $19.6–28.0 \times 3.6–5.6\,\mu$ (Av. 25 = $22.8 \times 4.9\,\mu$); $19.6–28.0 \times 5.3–9.8\,\mu$ (Av. 25 = $23.0 \times 7.0\,\mu$).

, The perfect stage was obtained by inoculating sterile grape stems in culture tubes with isolates of the fungus and overwintering the inoculated

stems out-of-doors.   Pycnidia, spermogonia, and ascocarp initials were pro-
duced in botryose stromata on these stems during late fall and winter.   All
three stages have been found associated in a single stroma.   Although the
pycnidia on berries were separate, those on the stems were usually grouped
in stromata and would be referred to *Dothiorella*.   Mature ascocarps were
produced in the stromata the following spring.   They were spherical and
measured 172–315 μ in diameter.   The locule was filled with a mass of
pseudoparaphyses and asci.   The asci were cylindrical, 8-spored, possessed
a thick, two-layered wall, and measured 102.4–156.8 × 17.6–24.0 μ.   The
ascospores were hyaline, unicellular, ovoid to elliptical, and measured 19.6–
30.8 × 8.4–11.2 μ (Av. 25 = 24.9 × 10.3 μ).   Isolates derived from ascospores
were identical with those obtained from rotted berries bearing the *Macro-
phoma* pycnidia.

The relationship of the *Botryosphaeria* perfect stage on over-wintered
stems with the *Macrophoma* imperfect stage associated with berry rot is
based on the morphologic connection of the pycnidia and ascocarps on grape
stems and upon the fact that isolates from both sources are similar in culture.
Inoculations have been inconclusive because of natural infection of berries
in the field with both *Botryosphaeria ribis* and *Melanconium fuligineum*.—
E. S. Luttrell, Georgia Experiment Station, Experiment, Georgia.

BOOK REVIEW

WOLF, F. A. and F. T. WOLF. *The Fungi.* Volume I. i–viii and 438 pp., 153 figs.; Volume II. i–xxii and 538 pp., 82 figs. John Wiley and Sons, New York; Chapman and Hall, Ltd., London. 1947. Vol. I, $6.00; Vol. II, $6.50.

This treatise on fungi will serve, as the authors intended, for both a text book and a reference. It will probably fill most of the requirements of the undergraduate student and the teacher. It will also be valuable to the research worker, who, through excessive specialization, may have been unable to keep up with recent mycological advances. The subject matter is attractively presented, is easy to read, and is abundantly illustrated. The occasional quip or jest appearing in the text adds zest to the reading. Each chapter contains a résumé of the matters just discussed, or a summary of relevant implications.

Volume I opens with a short chapter concerned with the history of mycology. This is followed by an outline of modern isolation and culture methods. Of interest to most readers and of great value to the student is a chapter dealing with the principles of fungal taxonomy and classification. The remainder of the volume consists of chapters individually dealing with Myxomycetes, Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti. For each class of fungi, the authors provide a description of its distinguishing features supplemented by descriptions of the principal orders and families. To some the lack of generic keys may seem a fault, but, since attention is drawn to detailed monographic studies dealing with individual groups of fungi, this fault is not serious. In any case, the inclusion of detailed keys would unnecessarily add to the volume and cost of the treatise.

Volume II, devoted to an account of the various activities of fungi, appears to be more suitable for the advanced student of fungi than for the undergraduate. The subject matter consists of chapters dealing with fungal nutrition, enzymatic action, respiration, biochemistry, and the effects of temperature, radiation, reaction of substrate, and association. Detailed accounts are given of spore dissemination and germination, host penetration, and physiologic specialization and variation. In other chapters, the authors discuss such subjects as mycorrhizae, genetics of fungi, poisonous and edible fungi, medical mycology, geographic distribution of fungi, mycology in relation to plant pathology, soil fungi, fungus-insect relationships, marine fungi, and fossil fungi. Many of these subjects are seldom dealt with in other texts and their inclusion adds interest and value to the volume. Not to be overlooked is the excellent author and subject index in both volumes.

It is apparently extremely difficult, in a monumental work of this kind, to avoid the intrusion of at least some errors and only the principal ones are referred to here. For instance, the alternative use of "swarmers," "swarm spores" and "zoospores" without an indication of synonymy may lead to some confusion of the uninitiated reader. Similarly, the persistent misspelling of the names Christensen and Rodenhiser in both text and citations may put difficulties in the way of a student who attempts to locate these authors in library indices. The occasional misspelling of other surnames is obviously a typographical error. The illustrations, though good, are occasionally placed too far away (once 6 pages and once 10 pages away) from the subject discussed in the text. In the discourse on the genetics of fungi, an illustration said to show sex segregation in the second nuclear division actually illustrates segregation during the third division. *Morchella esculenta,* initially referred to as a desirable edible fungus, is included, figured, and described among notoriously poisonous fungi. A considerable number of lesser errors appear, but to the reader they are not misleading and will no doubt be corrected in further editions.

It is problematical whether the average student can afford the price charged for each volume. In the light of the intrinsic value of the treatise and the present cost of printing, the price may not be excessive. The cost may be, however, prohibitive to the student, especially when his other necessary book expenses are taken into account.—JOHN E. MACHACEK, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.

SPORULATION BY PIRICULARIA ORYZAE[1]

B. W. HENRY AND A. L. ANDERSEN[2]

INTRODUCTION

During research on the relation of environmental factors to the development of the "blast" disease of rice caused by *Piricularia oryzae* Cav. (1), conidia were needed in quantity for use as inoculum in the various experiments. It was soon evident that sporulation by *P. oryzae* is a process distinct from mycelial growth in many respects. A certain amount of vegetative growth is of course prerequisite to the formation of conidia, but did not always lead to sporulation. Miller (13) has recently recorded similar findings with other plant pathogenic fungi. Tochinai and Shimamura (19), Konishi (8), and Aoki (2), reported physiologic specialization in *P. oryzae* using the degree of sporulation in culture as one characteristic. The present paper describes the process of spore formation by *P. oryzae*, the effects of some factors upon sporulation, and the relation of sporulation to macroscopic characters.

MATERIALS AND METHODS

Subcultures of a strain of *Piricularia oryzae* furnished by Dr. E. C. Tullis,[3] or of its reisolates obtained by plant passage, were used in all the reported experiments. Temperature effects on sporulation were studied with the aid of incubators adjusted to $\pm 0.5°$ C. The standard substrates used were 2 per cent rice-polish agar, and a 1:1 mixture of oats and Hegari sorghum seed plus water equal to 1.15 times the dry weight of the mixture. The comparison of these with other substrates is discussed later. Culture vessels for the whole-seed substrates were of three types. One consisted of 3 wire-screen trays in an enameled photographic pan $16\frac{1}{2} \times 9\frac{1}{2} \times 1\frac{1}{2}$ inches, with another pan inverted over the top. A $\frac{1}{2}$-inch opening was left at one end by supporting the top pan on cork stoppers. The trays, each $13\frac{1}{2} \times 7\frac{1}{2}$ inches, were tiered inside the resulting semi-closed chamber by supporting the corners of each tray on cork stoppers coated with paraffin. About $\frac{1}{4}$ inch of sterile water was kept in the bottom pan. To forestall contamination by air-borne organisms, the substrate samples were sterilized, inoculated, and incubated for 3 or 4 days in quart milk bottles plugged with cotton, prior to placing them on the trays. The photographic-pan type of vessel was used

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for some of the initial studies on the different grain substrates and for the production of quantities of spore inoculum. It was soon supplanted, however, by the other types.

The second type of vessel was a galvanized-iron chamber $18 \times 12 \times 16$ inches with a sliding panel door and contained ten removable copper-screen trays (Fig. 1, A). The entire chamber and aeration attachments (exclusive of the manometer) were autoclaved for one hour at 20 pounds steam pres-
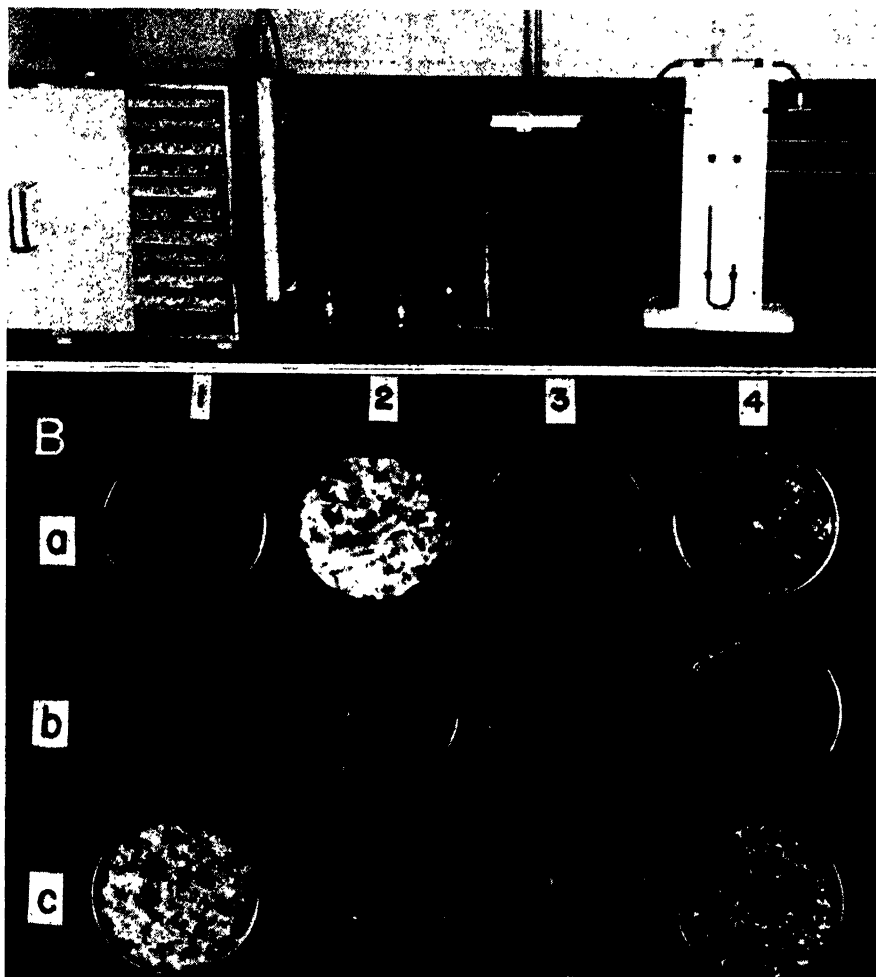
FIG. 1. A. Growth chamber for studying sporulation on whole-seed substrates. B. Petri-dish cultures of *Piricularia oryzae* on oats-sorghum substrate showing variation in macroscopic characters. Cultures b–1 and b–3 produced conidia in abundance; cultures a–2 and c–1 were nearly sterile; others were intermediate.

sure. One 200-gm. or two 100-gm. dry wt. samples of substrate were used per tray. As with the photographic-pan type of vessel, the seeded substrate was incubated in quart milk bottles prior to placing it on the trays. The chamber was supplied with filtered, humidified air at a controlled rate meas-

ured by the manometer in the air line.   To increase the number of samples
that could be tested, the chamber was also used as an incubator for Petri-dish
cultures.   Fifteen-gm. dry wt. samples of substrate were sterilized, inocu-
lated, and incubated for 2 to 3 days in 9-cm. Petri dishes.   Twelve dishes
were then placed on each tray, the lids removed, and the incubation period
completed.   It was thus possible to test 120 samples at one time in the cham-
ber.   Since four replications were desirable, 30 isolates or 30 substrates could
be tested simultaneously.   This type of vessel was used for studies on the
different grain substrates, the different isolates of *Piricularia oryzae*, the
effect of inoculum concentration, and for producing spore inoculum in
quantity.
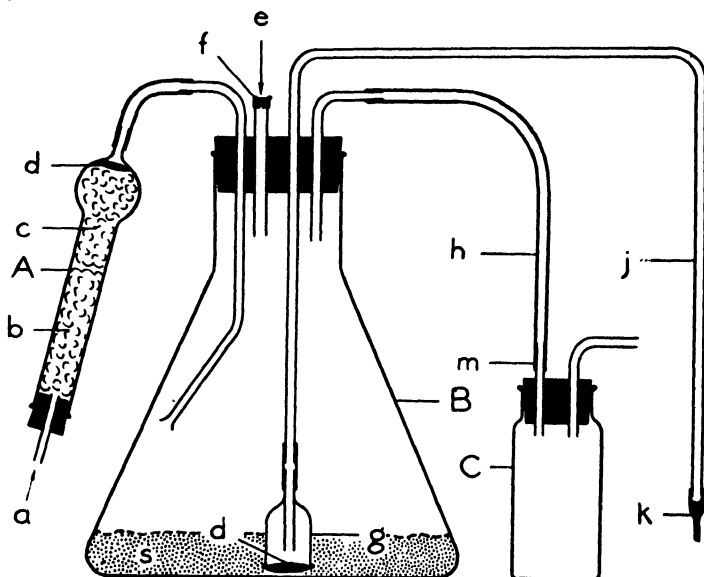
FIG. 2.   Flask with apparatus for aseptic production and removal of conidia.   A.
Air filter.   B. The 3-l. Fernbach flask.   C. Air trap.   a. Air inlet.   b. Glass wool. c.
Absorbent cotton.   d. Copper screen.   e. Inoculation tube.   f. Serum bottle cap.   g. Filler
tube.   h. Air outlet and water inlet.   j. Suspension exit tube.   k. Rubber policeman.
m. Rubber hose connection to facilitate the removal of air trap (C) prior to the introduc-
tion of water through (h) at time of harvest.   s. Culture on whole-seed substrate.

A third type of culture vessel, for studies on whole-seed substrates, *e.g.*,
rate of aeration, period of incubation, etc., was a large-mouth, 3-l. Fernbach
flask equipped for forced aeration, for the aseptic introduction of sterile
water, and for the aseptic removal of the spore suspension (Fig. 2).   A
200-gm. dry wt. sample of substrate was used.   The spores were put into
suspension by shaking the flask in a rotary manner, and the suspension was
forced out through the "filler tube" by slight air pressure.   For experiments
in which the spores did not need to be harvested aseptically, only that equip-
ment needed for forced aeration was employed.

Spore suspensions for seeding all cultures were obtained by adding sterile
water to 5- to 7-day-old agar cultures in tubes and lightly scraping the sur-
face with a wire loop.   Agar media in test tubes were seeded by spreading

a 5-mm. loopful of the suspension over the surface of the agar. Whole-seed substrates were seeded by aseptically applying a given amount of the suspension from a hypodermic syringe and distributing it by gently rotating the culture vessel. Spore yields and spore density of inocula were determined with the aid of a "Howard Mold Counting Chamber, modified type" (Arthur H. Thomas Co., Philadelphia, Pa.).

THE PROCESS OF SPORE FORMATION

The sporulation process of *Piricularia oryzae* was studied in detail on water and rice-polish agars using hanging-drop cultures, agar films on microscope slides, and cleared agars in Petri dishes. The conidia were obclavate to pyriform, pale olive to colorless, usually 3-celled, and approximately $8-9 \times 20-26$ microns. They were formed on conidiophores morphologically similar to the vegetative hyphae. At first a single conidium formed at the apex of a conidiophore (Fig. 3, A). Later a branch grew from the conidio-
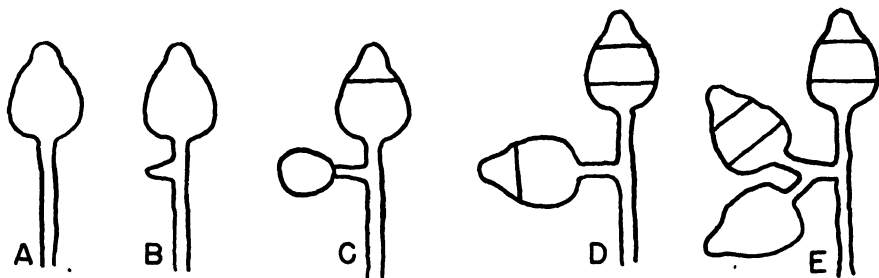
FIG. 3. Diagrammatic sketches of the method of conidium formation by *Piricularia oryzae*. Details are given in the text.

phore a few microns below the point of attachment of the first spore, and another conidium was formed at its apex (Fig. 3, B, C, and D). A second branch appeared a few microns below the point of attachment of the second spore and the third conidium was formed (Fig. 3, E). This process of sympodial branching continued; as many as 9 conidia were observed on 1 conidiophore. General observations of sporulation on individual seeds of oats and sorghum, and on host lesions confirmed the detailed studies. These data are in general accord with those of Aoki (2), and indicate that the process of spore formation by *P. oryzae* in artificial culture is like that occurring on the host.

FACTORS AFFECTING SPORULATION
Concentration of Inoculum

In studying the growth and metabolism of fungi in culture, it is usually considered that, within quite wide limits, the concentration of spore inoculum is not a determining factor (11, 15). The incubation period required for the production of certain metabolic products by fungi in liquid culture has been reportedly shortened by using heavier spore inoculum (5, 22). No reported work on the effect of this factor on conidia production has come to the authors' attention. In order to standardize the procedure for further

experiments, the effect of inoculum concentration on spore yield was determined for both the agar and the whole-seed substrates.

Rice-polish-agar slants were seeded with suspensions having 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, and 4.0 million spores per ml. Spore yields were determined after 7 days' incubation at 25° C. The inoculum containing 0.01 million spores per ml. gave a yield of 14 million spores per tube, and all higher concentrations yielded 20 to 24 million spores per tube, except the concentration of 2.0 million per ml. which gave 29 million per tube. Further experiments failed to show the peak with the 2.0 million per ml. concentration. It was concluded that a 5-mm. loopful of a suspension of 0.05 million or more spores per ml. was optimum for seeding the agar slants.

One-hundred-gram units of oats-sorghum substrate in quart milk bottles were each seeded with 5 ml. of inoculum and transferred to the growth chamber after 4 days' incubation at 25° C. The inoculum concentrations were 5, 20, 100, 200, and 300 thousand conidia per ml.; the yields, after a total of 7 days' incubation, were 2.5, 6.5, 6.0, 8.0, and 8.0 million spores, respectively, per gm. of substrate. Thus the optimum concentration of inoculum for this substrate seemed to be 200,000 conidia per ml. Further experiments confirmed this optimum and demonstrated that the higher concentrations or greater volumes of inoculum were not beneficial.

The optimum concentrations of inoculum given above for agar and whole-seed substrates were used throughout later experiments.

Substrates

Piricularia oryzae was studied on agar media in quadruplicate series of 8-ml. lots slanted uniformly in 18 × 150 mm. Pyrex test tubes. The spore yields were determined after 5 to 8 days' incubation at 24° C. Nineteen synthetic and 52 nonsynthetic agar media were tested.

The synthetic media included various sugars as carbon sources, inorganic and organic nitrogen sources, magnesium sulphate, potassium phosphate, agar, distilled water, and, in some cases, thiamin. There was no appreciable growth and no sporulation on any of these agars. The synthetic medium reported by Tochinai and Nakano (19) as supporting good growth and sporulation by *Piricularia oryzae* failed to support growth of our isolates. It was later shown (9) that both thiamin and biotin were required for the growth and sporulation of the isolates used in these studies.

The nonsynthetic agar media were made from many vegetables and cereals as well as several commercially prepared materials, *e.g.*, malt extract, yeast extract, beef extract, peptone, "Cerophyll," etc. All the nonsynthetic media supported growth of *Piricularia oryzae* to some degree. The requirements for sporulation were evidently more exacting. Yeast-extract, peptone, and barley-extract agars all gave good vegetative growth but no sporulation. Rice-polish, rice-bran, dried-green-rice-tissue, "Cerophyll," wheat, sorghum, oats-sorghum, and Bacto-malt agars gave good vegetative growth and abundant sporulation. It was evident that the luxuriant development of my-

celium was not always accompanied by the production of conidia, whereas the abundant production of conidia was always accompanied, or preceded, by considerable growth of mycelium. Rice-polish and rice-bran agars gave in general the highest spore yields, and the former was selected as the active ingredient in the stock culture medium. It was originally used at a 3 per cent concentration but later trials showed 2 per cent to be as good for sporulation, so this concentration was adopted. Representative data showing the quantity of sporulation by 5 isolates of *P. oryzae* on 3 nonsynthetic agars are presented in table 1. It was evident that the different media had a dissimilar influence on the quantity and stability of sporulation by the various isolates.

TABLE 1.—*Sporulation by 5 isolates of Piricularia oryzae subcultured at 5-day intervals on 3 different agars*[a]

Isolate[b]	2 per cent rice-polish agar					20 per cent sorghum agar					20 per cent oats-sorghum agar				
	Subculture					Subculture					Subculture				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	7.7	10.6	6.7	5.6	9.6	1.7	6.5	4.7	4.8	5.1	1.3	3.3	3.5	4.7	10.3
17	4.2	6.7	9.7	3.2	1.9	0.8	3.4	3.0	2.1	3.7	2.5	3.1	2.0	1.5	1.6
27	10.1	11.2	6.1	3.1	1.2	3.7	2.9	1.6	0.7	0.3	3.4	2.3	0.4	0.0	0.1
42	25.7	7.1	6.8	11.0	18.5	0.9	3.8	5.5	8.4	9.9	4.5	2.7	4.5	2.2	8.1
54	14.8	7.5	4.7	1.3	0.4	0.7	1.9	1.8	2.3	3.9	1.9	1.5	1.5	0.7	1.1

[a] Data presented in millions of conidia per test tube culture. Each figure represents the average of 3 cultures.
[b] Isolate 1 was a subculture of the original strain. The other 4 isolates were obtained by host-passage of isolate 1.

Sporulation by *Piricularia oryzae* was studied on several whole-seed substrates in triplicate series of 100-gm. dry wt. samples plus the maximum amounts of water that would be imbibed during autoclaving. The seeded substrates were incubated at room temperature for 4 days in quart milk bottles and then transferred to the screen trays in photographic pans for an additional 4 days. Wheat, corn, oats, soybeans, cowpeas, and Hegari sorghum seeds were used separately. *P. oryzae* grew on all these substrates, with the most abundant development of mycelium on soybeans and cowpeas. The yields in millions of spores per gm. dry wt. of substrate were: wheat, 0.7; corn, 0.4; oats, 2.4; soybeans, 0.0; cowpeas, 0.0; and sorghum, 4.8. As with the agar substrates, abundant growth of mycelium did not ensure abundant production of conidia, since the two substrates which gave the most mycelial growth, namely, soybeans and cowpeas, yielded no conidia. It was evident that sorghum seed was the best of the group for the production of conidia. This substrate had the disadvantage however that the grains clumped together, presumably because of rupture during autoclaving. In an attempt to alleviate this difficulty, several materials of possible value as nutrient sources or as fillers, were mixed in various proportions with sorghum seed. Among these materials, rice hulls, peanut hulls, and the seeds

of oats, wheat, or corn aided in preventing the clumping of the sorghum; rice hulls and oats gave mixtures with the best physical qualities. The mixtures containing peanut hulls, rice hulls, and wheat all gave lower spore yields than sorghum alone, while mixtures containing certain proportions of oats and of corn gave higher yields than sorghum alone. The following are representative yields of the oats and corn mixtures in millions of conidia per gm. dry wt. of substrate: sorghum alone, 8.8; oats-sorghum at 6:5, 7.0; oats-sorghum at 1:1, 16.0; oats-sorghum at 3:5, 11.0; corn-sorghum at 6:5, 6.0; corn-sorghum at 1:1, 8.2; corn-sorghum at 3:5, 14.0. The 1:1 mixture of oats-sorghum was the best of the grain substrates from the standpoint of sporulation and also possessed the desired physical qualities. It was thus adopted as the substrate for production of conidia in quantity.

Aeration of Cultures

Piricularia oryzae produced conidia in abundance on suitable agar media in test tubes plugged with cotton. When cultured on agar in larger vessels, such as Roux flasks, relatively few or no conidia were produced even though other conditions were the same; likewise, few conidia were produced when the fungus was grown on 200-gm. dry wt. samples of a whole-seed substrate in 3-l. Fernbach flasks plugged with cotton, or in other semi-closed vessels. Brown (3) has reported ammonia to be a staling product produced by some fungi, and Mader (12) reported inhibition of mushroom fructification associated with a staling of the atmosphere about the cultures. After a few days' incubation, an ammonia-like odor could often be detected about the larger cultures of *P. oryzae* and wet litmus paper held in a culture vessel turned blue. These data indicated that there was a staling of the air about the cultures.[4] A series of experiments on the effect of forced aeration was begun.

The fungus was grown on sorghum seed in 3-l. Fernbach flasks equipped for the introduction of measured volumes of sterile, humidified air (Fig. 2). The rate of aeration was calculated in cc. of air/min./gm. of substrate. The effect on spore yields of rates up to 4.0 cc. are shown graphically in figure 4, A. It was evident that the rate of aeration had a pronounced effect upon sporulation and that the range of 0.0 (flask plugged with solid rubber stopper) to 4.0 cc./min./gm. did not go high enough to demonstrate the optimum. Sorghum was again used in one experiment and a 1:1 mixture of oats and sorghum was used in another in which higher rates of aeration were included. In both experiments, the sporulation curve levelled off at aeration rates of 3.3 to 4.0 cc./min./gm., and at about 5.0 cc./min./gm. or above sporulation was decreased. The latter may have resulted from partial drying of the substrate by the blast of air. The rate of 4.0 cc./min./gm. was concluded to be optimum for the grain substrates in Fernbach flasks.

[4] E. C. Tullis and A. L. Andersen (unpublished data) had previously obtained similar results with *Piricularia oryzae*.
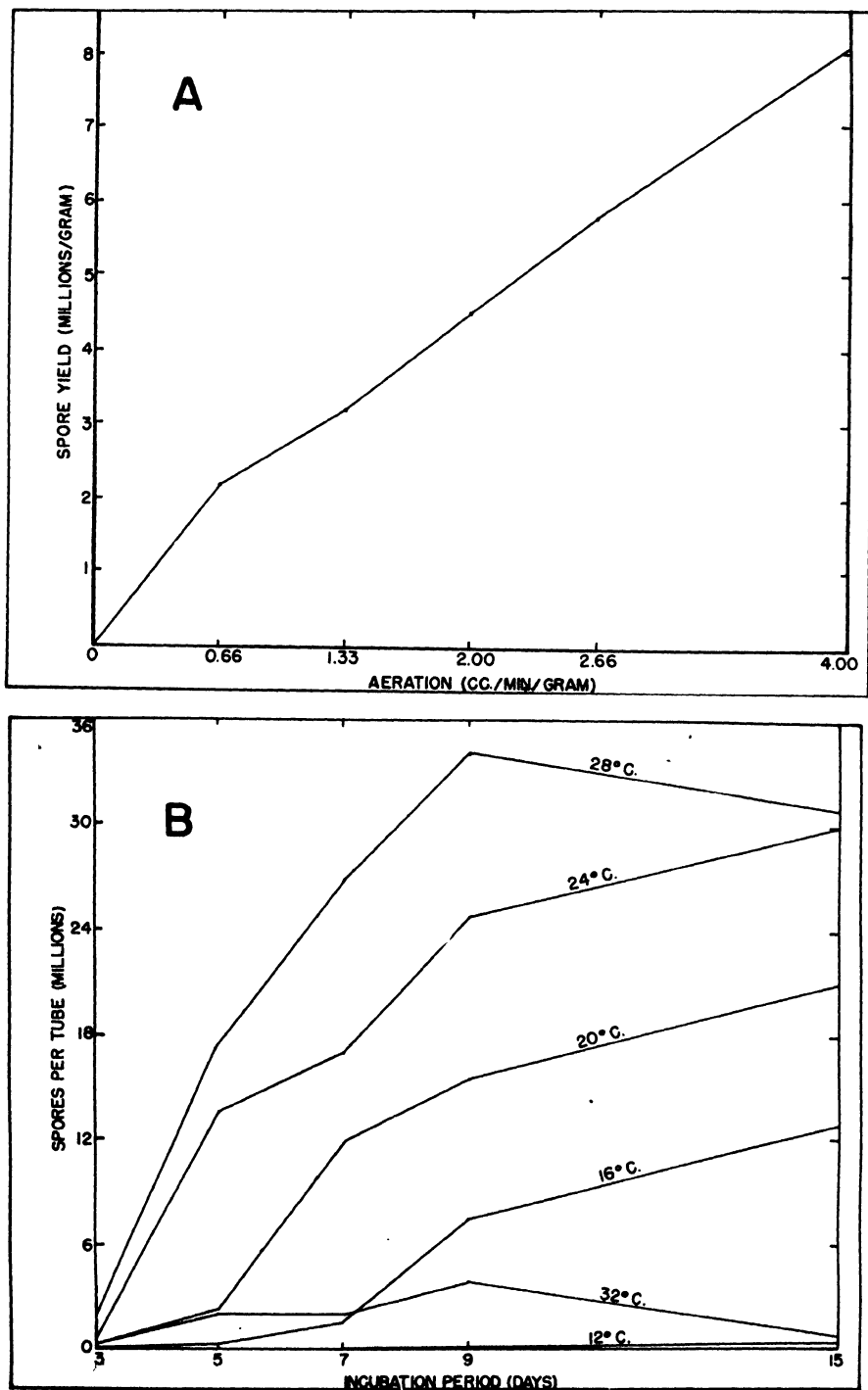
FIG. 4.   A. The effect of aeration on sporulation by *Piricularia oryzae* on sorghum-seed substrate.   B. The effects of temperature and time of incubation on sporulation by *P. oryzae* on rice-polish agar.

Moisture Content of Air

Hemmi and Imura (7) reported that conidia were produced by *Piricularia oryzae* on host-lesions in air maintained at 93 per cent or higher relative humidity. Andersen, Henry, and Tullis (1) concluded that high relative humidity was necessary for the production of conidia on the host. In the present studies, observations have shown that the air must be maintained at a high moisture content to obtain maximum sporulation on grain substrates. In practice, therefore, the air was humidified by bubbling it through sterile water. By governing the height of the water column and the size of the air bubbles it was possible to maintain the desired humidity without excessive condensation of moisture.

Moisture Content of Substrate

It was evident early in the course of these studies that high moisture content of the whole-seed substrates favored sporulation by *Piricularia oryzae*. It was desirable to incorporate the maximum amount of water that could be imbibed by the substrate without excessive bursting of the individual kernels. For the preferred oats-sorghum mixture, this amount was 1.15 times the weight of the dry grain. The two components of the substrate imbibed about equal amounts of the added water. If the water was added to the grain immediately prior to autoclaving, the result was excessive bursting of the sorghum kernels near the bottom of the layer of substrate with the upper part remaining too dry. This difficulty was alleviated by steeping the grain in hot water prior to autoclaving it. The desired amount of water was taken up by the oats-sorghum mixture in a 2-hour steep in an excess of water at 82° C., or in a 4-hour steep at 76° C. A less refined but faster method of steeping was to add the amount of water to the grain in individual culture vessels, heat the whole in an autoclave for about 15 min. at 107° C. and 3 to 5 lb. pressure, and remove and shake it while still hot. Sterilization of the substrate was accomplished by autoclaving in the usual manner. The latter method of steeping was used for most of the reported studies.

Time of Incubation

The effect of time of incubation upon sporulation by one isolate of *Piricularia oryzae* on rice-polish agar is shown in figure 4, B. Maximum sporulation at the optimum temperature (28° C.) was reached by the 9th day in this case. The sigmoid-type curve shows the most rapid sporulation to have taken place between 3 and 5 days after seeding, with a diminished rate from 5 to 9 days. Another isolate of *P. oryzae*, a comparatively poor sporulator, showed the same period of most rapid sporulation, but there was no further increase after 5 days. The time required for maximum sporulation may thus differ between isolates.

The time required for maximum sporulation on the oats-sorghum substrate varied with the way in which the cultures were handled. When they were incubated in milk bottles prior to being placed in aerated vessels, maxi-

mum sporulation was obtained after 4 days in the latter vessel, or a total incubation period of 7 to 8 days. Maximum sporulation was reached by the 6th day in cultures incubated in aerated Fernbach flasks.

Temperature of Incubation

The effect of temperature on sporulation by *Piricularia oryzae* was studied by incubating seeded tubes of rice-polish agar in cabinets held at 4° steps from 4° to 36° C. The amount of sporulation was determined at intervals up to 15 days (Fig. 4, B). Spores were produced most rapidly and in greatest quantity at 28° C. with the maximum yield of 34 million per tube. Spore production at 24° C. was not so rapid as at 28° C. but nearly equalled the latter after 15 days' incubation.

It is probable that the amount of sporulation at 16° and 20° C. would have reached the maximum set at 28° C. if the incubation period had been extended. There was no growth or sporulation at 4° or 8° C. The organism was not killed however, as some tubes removed from these temperatures after 9 days and incubated for an additional 6 days at 24° C. gave spore yields of approximately the same magnitude as the 6-day point on the 24° C. curve. There was good growth but little sporulation at 32° C. and slight growth but no sporulation at 36° C. The viability of the spores produced at 12° to 32° C. was unaffected but those produced at 32° C. developed short, knobby, abnormal germ tubes. Therefore, the optimum temperature for the production of conidia by *Piricularia oryzae* appeared to be 28° C. as reported by Tochinai and Shimamura (20). This temperature is also the optimum for mycelial growth as reported by Nisikado (14), and confirmed by Yoshii (23) and the present studies. Thus *P. oryzae* is similar to many other fungi in having the same optimum temperature for growth and sporulation (10, 21). At 28° C., however, sporulating isolates tended to give rise to white, asporogenous forms. Similar results with two species of *Magnusia* were reported by Sweet (18). To reduce this danger, the next lower temperature, 24° C., was used routinely for the incubation of all cultures.

Initial pH of Substrate

The effect of the initial pH of the substrate on sporulation by *Piricularia oryzae* was studied by incubating cultures on adjusted rice-polish agar at 24° C. and determining the number of spores produced per tube after 7 days. The various pH values were obtained by adding 0.1 N HCl or NaOH to the agar samples after autoclaving and in amounts determined from a titration curve prepared for rice-polish agar. The actual value of an aliquot sample in each case was determined with a Beckman pH meter. A pH range of 3.0 to 8.8 was used. Growth and sporulation occurred from 4.1 to 8.8 with maximum sporulation between 4.9 and 7.5. There was no growth on media more acid than pH 4.1. Nisikado (14) reported growth of *P. oryzae* throughout the pH range of 5.0 to 10.0. On the basis of these data, the two standard

substrates, rice-polish agar, pH 5.8 to 6.2, and 1:1 oats-sorghum substrate, pH 6.8, fell naturally within the optimum pH range for sporulation.

Frequent Subculturing

Twenty-five mass isolates and 25 single-spore isolates of *Piricularia oryzae,* all progeny of the original strain, were transferred weekly to fresh tubes of rice-polish agar for 9 weeks. At the end of selected incubation periods, the average spore yield for three tubes of each isolate was determined. Representative data are given in table 2. The results show a considerable amount of variation. The amount of sporulation increased in some isolates, decreased in most, and fluctuated in all during the 9-week period. The mass isolates and single-spore isolates were alike in this respect.

TABLE 2.—*Sporulation by representative isolates of* Piricularia oryzae *subcultured weekly for 9 weeks on rice-polish agar*[a]

Isolate	Subculture					
	1	2	3	5	7	9
42	10.0	16.0	8.3	8.0	5.0	3.6
42–A[b]	8.0	11.3	9.7	5.8	5.8	9.0
42–B[b]	2.3	1.1	1.4	2.7	2.9	1.6
42–C[b]	7.3	14.6	11.6	8.0	7.0	7.2
42–D[b]	2.5	1.4	2.7	4.3	2.9	1.2
42–1[c]	7.9	4.6	8.8	5.5	10.5	14.0
42–2[c]	11.4	10.5	11.6	2.3	2.4	0.7
42–3[c]	8.7	11.9	9.5	6.1	4.3	5.6
42–3–1[d]	.. .	. . ..	10.4	6.3	1.8	0.8
42–3–2[d]	....	. . .	9.7	7.2	2.4	1.3
42–3–3[d]	. . . .	.	12.8	8.6	2.6	5.3

[a] Data in millions of conidia per test-tube culture. Each figure represents the average of 3 cultures.
[b] Reisolates obtained by host-passage of isolate 42.
[c] Single-spore isolates from isolate 42.
[d] Single-spore isolates from isolate 42–3.

Other high-sporulating isolates kept as stock cultures were subcultured frequently on rice-polish agar for periods up to 15 months. The amount of sporulation fluctuated considerably between subcultures, but the general tendency over a long period was always toward decreased sporulation. Greene (6) reported a similar tendency in high-sporulating cultures of *Aspergillus fischeri.*

Storage of Isolates at Low Temperatures

Transfers of all isolates of *Piricularia oryzae* used in these and allied research studies were stored in tubes of rice-polish agar at 6° to 8° C. Some cultures were untreated and others were covered with a layer of sterile mineral oil (16). Cultures stored with or without oil were viable for at least 13 months. Fresh transfers of most cultures that were stored under oil for 13 months gave higher sporulation than fresh transfers of like cultures that were stored without oil. Cultures better retained their ability to

sporulate when stored at 6° to 8° C., with or without oil, than when they were frequently subcultured at 24° C. over a like period of time.

Host Passage of Isolates

About 100 different isolates of *Piricularia oryzae* were obtained by plant passage of the original strain. The amount of sporulation by subcultures of many of these on rice-polish agar was studied. The variation in the amount of sporulation between and within these isolates was great (See tables 1 and 2). Within a group of isolates obtained from lesions on identical plants that had been inoculated simultaneously with the same lot of inoculum, there appeared some that sporulated more and some that sporulated less than the isolate used as inoculum. The magnitude of the difference varied between groups. Repeated host passage of a particular isolate did not seem to lessen the variation in sporulation between or within its reisolates. In spite of the tremendous amount of variation, it has been possible to retain the high-sporulating characteristic of isolates of *P. oryzae* for 3 years by means of host passage.

RELATION OF SPORULATION TO MACROSCOPIC CHARACTERS

The observation of macroscopic characters accompanied the study of sporulation by *Piricularia oryzae*. A so-called normal colony of *P. oryzae* was olive-gray with aerial hyphae to a height of 1 to 3 mm. Most of the high-sporulating isolates were of this type, but few were darker with more appressed aerial growth. The non-sporulating isolates were much lighter to nearly white, with a 3 to 10 mm. fluffy layer of aerial hyphae. There were all gradations between the two extremes. The original strain was of the normal, sporulating type, and gave rise to all the other types encountered in this study. The non-sporulating types arose in different ways, such as incubation at 28° to 32° C., frequent transfer on rice-polish agar, growth on peptone, yeast-extract, and barley-extract agars, and on seeds of soybeans and cowpeas. There was also the appearance, in some cases, of a non-sporulating patch sector not evidently associated with any of these environmental conditions. Such sectors may have been mutants. Stevens (17) and Christensen (4), working with *Helminthosporium sativum* P. K. and B., reported that pale, poor-sporulating mutants occurred most frequently. Figure 1, B, illustrates the macroscopic appearance of some *P. oryzae* isolates on oats-sorghum substrate.

DISCUSSION

Sporulation by *Piricularia oryzae* is a process influenced by several factors. Given an isolate inherently capable of sporulating, the first requisite is a certain amount of vegetative mycelium. Those factors basically necessary for the production of the vegetative thallus might therefore be listed as necessary for sporulation. Some of these factors, such as pH of the substrate and temperature of incubation, seem to affect sporulation largely as

a result of their effects on vegetative growth. The optimum state of such factors appears to be about the same for the growth and sporulation processes. Other required factors, such as the specific substrate, the moisture content of the substrate and the adjacent atmosphere, and aeration of the culture, may meet the requirements for vegetative growth but be unsuitable for sporulation. Forced aeration, for example, did not seem to be required for vegetative growth of *P. oryzae* under the experimental conditions, but was required for sporulation in culture vessels larger than the ordinary test tube. Certain substrates, such as whole-seeds of soybeans or cowpeas, supported luxuriant development of mycelium but no sporulation, thus indicating that nutrients and growth factors in quality and quantity favorable for vegetative development may not be favorable for spore development. Hence the requirements for sporulation by *P. oryzae* are more exacting, and possibly more numerous, than the requirements for growth.

SUMMARY

The process of spore formation by *Piricularia oryzae* in artificial culture was found to be similar to that on the host.

High-sporulating isolates were usually olive-gray with a 1 to 3 mm. layer of aerial hyphae. The relatively nonsporulating isolates were a lighter color to nearly white, with a 3 to 10 mm. layer of aerial hyphae.

High-sporulating isolates were best retained in that condition by storage under mineral oil at 6° to 8° C.

Of the several agar and whole-seed substrates suitable for spore production, the best were 2 per cent rice-polish agar and a 1:1 mixture of oats-sorghum, respectively. Spore yields were increased with increased concentrations of inoculum up to an optimum range.

Moisture contents of near saturation for the whole-grain substrates and the adjacent atmosphere favored abundant sporulation.

Forced aeration was necessary for sporulation in culture vessels larger than the ordinary test tube. A rate of about 4 cc. of air/min./gm. of oats-sorghum substrate was optimum.

The length of the incubation period required for highest sporulation varied with the temperature, the culture vessel, and, to some extent, with the isolate.

The effects of incubation temperatures and pH of the substrate on sporulation appeared to be a result of their effects on growth. The optimum state of these factors, 28° C. and pH 4.9 to 7.5, appeared to be about the same for growth and sporulation.

Isolates of the pathogen, obtained by host-passage of the original strain, followed in some cases by single-sporing, varied considerably in the quantity of spores produced in a given environment.

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AN ELECTRON MICROSCOPE STUDY OF TOBACCO MOSAIC VIRUS EXTRACTED FROM PULP AND JUICE AFTER VARIOUS PERIODS OF INFECTION

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The mode of virus multiplication or increase is among the most interesting problems in pathology. An earlier attempt (2) to gain evidence on this process by studying the length of virus particles in juice from leaves infected for various periods was described in 1946. Bawden (1) has reported that tobacco mosaic virus obtained from finely macerated leaf pulp has shorter particles than does virus obtained from juice. In the work described here we have tried to get the virus from finely macerated pulp as well as from the juice and have avoided the use of ultracentrifugation, chemical treatments, or filtration which may cause aggregation or loss of virus. It was hoped that an electron microscope study of combined pulp and juice virus obtained at intervals after infection might provide evidence regarding the changes that occur in virus particles during multiplication or increase.

METHODS

Unfrozen tobacco leaves which had been infected for various periods of time were finely ground in a hand operated ground glass homogenizer to try to free the virus in both pulp and juice. One gram fresh weight of leaf blade was used for each sample. Five cc. of distilled water were added during the grinding to produce a liquid suspension of the pulp and juice. This mixture had the pH of plant juice, around 5.6. The mixture was then centrifuged at about 2500 r.p.m. for 1 hour to remove the coarser material. The supernatant was diluted with distilled water and placed on celloidin membranes for electron microscopy.

RESULTS

As in earlier work (2) it was found that the lengths of tobacco mosaic particles usually fall into 3 fairly distinct groups. The length distribution curves usually show a group of the shortest particles having lengths under 225 mμ, a group of medium length particles having lengths between 225 and 463 mμ, with a rather high peak around 300 mμ, and a third group composed of the longest particles having lengths greater than 463 mμ. In the length distribution curves the middle group is usually separated from the other groups by distinct valleys indicating that the particles in the shortest and longest groups are not simply variants in a normal distribution about the middle group.

The proportion of the particles in each group after various periods of infection are shown in table 1. The results indicate that the proportion of

the particles in the shortest group becomes greater as multiplication proceeds between the fourth and sixteenth day after inoculation of the leaf. The proportion in the middle group did not show any distinct change with increasing age of infection.

TABLE 1.—*Proportion of virus particles in each size group when extracted at pH 5.6 at intervals after infection*

Experiment no.	Age of infection in days	No. of particles measured	Percentage shorter than 225 mμ	Percentage between 225 and 463 mμ long	Percentage longer than 463 mμ
31	4	124	11	43	44
34	4	156	15	43	42
34	6	150	10	14	74
31	6	79	23	40	37
33	7	529	20	69	9
34	9	290	31	54	14
33	12	394	35	60	6
34	12	330	32	37	31
31	13	365	40	37	23
34	16	1022	65	30	5
31	20	234	45	38	17
33	20	709	44	51	5
34	20	722	60	32	7

The proportion in the long group showed some tendency to decrease after the first six days of infection but the results showed considerable variation.

DISCUSSION

Perhaps the most interesting result obtained is the apparent increase in the proportion of particles in the short group during the process of virus multiplication. The combined results of Stanley (5), Spencer (4), and Rawlins, Roberts, and Utech (2) have given ample evidence to prove that there is a great increase in the number of middle group particles per cell during virus multiplication. Since the proportion of short particles apparently increases at the same time it may be concluded that there is also a great increase in the number of short group particles per cell during virus multiplication. Such a conclusion would be justified as long as there is no decrease in the proportion of short group particles during virus multiplication. The grinding of purified middle group particles from juice with the ground glass homogenizer has failed to change the length of the particles or to decrease their infectivity. The same result was obtained when the middle group virus was mixed with healthy pulp before grinding. These results apparently indicate that the increase in the proportion in the short group did not result from the action of the homogenizer on middle group particles. Since the results of Stanley (5) and Spencer (4) indicate that leaves infected for 20 days have from 3 to 7 times more middle group particles per cell than leaves infected for 7 days it is obvious that changes in the proportion

of particles in the 3 groups during virus multiplication may be due partly or entirely to different rates of increase of the 3 groups rather than to one group being derived from another. The results shown in table 1 therefore cannot be considered as evidence for or against the short group being derived by the breaking up of particles in one or both of the longer groups. Earlier work with juice virus (2) failed to show any distinct increase in the proportion of short group particles during virus multiplication. The increase shown in table 1 is therefore probably due to an increase in the proportion of short particles from pulp virus which was included in our samples along with the juice virus.

There is considerable uncertainty regarding the infectivity of the short group particles from pulp. Sigurgeirsson and Stanley (3) have given evidence that an unsedimentable fraction from infective plant juice, consisting mainly of short particles, shows little infectivity. They did not study the infectivity of the short particles from pulp. Some of our unpublished results indicate that about 30 per cent of the local lesions produced by virus extracted from pulp alone are produced by short group particles and that the other 70 per cent are produced by middle group particles. Other of our experiments indicate that all of the infectivity in the pulp virus is due to middle group particles. Accordingly more evidence must be obtained before conclusions can be reached as to whether all of the short particles from pulp are noninfective.

None of the results shown here or in the literature appears to give a satisfactory explanation of the origin of the short group particles or of the significance of this group in virus multiplication.

The mode of formation of the middle group also remains undetermined. All of our attempts to produce a distinct middle group by artificial aggregation of short group particles have been unsuccessful. This result apparently constitutes evidence against the middle group particles having been formed by the aggregation of short group particles.

Long group particles, on the other hand, may be readily produced at will by ultracentrifuging a mixture of short group and middle group particles in distilled water. Extraction of combined pulp and juice virus in phosphate buffer at pH 7 instead of distilled water prevents the formation of an appreciable number of long group particles. All of this evidence apparently indicates that the long group results by aggregation of middle group and short group particles when the pH is around 5.6. This tendency is particularly pronounced in juice from leaves which have been infected for 6 days or less.

SUMMARY

When tobacco mosaic virus is extracted from finely macerated pulp and juice at the pH of plant juice the proportion of short particles increases between the fourth and sixteenth days after infection. The concentration of short particles per cell, like that of middle group particles per cell, in-

creases during this period. The short particles did not result from fracture of middle group particles during maceration.

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THE SPECIES VALUE OF PATHOGENICITY IN THE GENUS XANTHOMONAS

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Elliott (5) has reviewed, from an historical point of view, the important changes in the classification and nomenclature of the bacterial plant pathogens. The changes brought about have been concerned primarily with disposition of the species of the outmoded genus *Phytomonas* into new or older genera emended to include the plant pathogens. For the moment, generic characters have been stabilized and White's (18) simple key for the working pathologist should be revised.

Keys to differentiate species within the genera are not too adequate. Burkholder (2) points out two contributing factors: (i) many of the species can be separated only on their pathogenicity to certain plants or groups of plants, *i.e.*, physiological tests are as yet inadequate and (ii) cross inoculations with the hosts of organisms of similar appearance and apparent physiological identity have not always been sufficiently complete to eliminate synonymy. With respect to these factors Burkholder urges a continued search for physiological tests of diagnostic value and points out the general areas where cross inoculation investigations might provide profitable information (2, p. 134). Stapp (12, p. 409) advocates "comprehensive comparative studies of the species . . . involving serological tests in addition to the usual study of morphological, cultural, and physiological characters."

Within the genus *Xanthomonas* of Dowson (4) numerous cross inoculation studies have been conducted. Those data, however, are scattered throughout the literature, but a glance through Elliott's *Manual of Bacterial Plant Pathogens* shows numerous species of plants susceptible to various organisms, which has been the result of cross inoculation work. It is a misfortune, in this instance, that she has not listed those plants that were not susceptible when tested. Furthermore, in the *translucens* group, Jones, Johnson, and Reddy (10), Hagborg (9), and Wallin (16) have made numerous cross inoculations among the cereals and have shown that phytogenic strains of the bacteria exist, not unlike those found in the rust fungi. In these inoculations recent isolates were used so that loss of pathogenicity of the various cultures could not account for the differences in the strains.

Progress has been reported too in the field of physiological tests. The contributions of Starr and Burkholder (14) and of Starr (13) point clearly to the fact that some species at present incorporated in *Xanthomonas* (*X. rubrilineans*) show few cultural characteristics relative to the genus as a whole, whereas others have exacting nutritional requirements, which may not be satisfied by synthetic basal media.

A recent paper by Elrod and Braun (6) presents a study of the genus by cross agglutination tests. These authors studied 36 species and subspecies of *Xanthomonas* and on the basis of their observations on cross agglutination reactions divide the species into five serological groupings as follows:

    I. The *campestris* group comprising *X. campestris* and *X. barbareae*.

    II. The *vascularum* group comprising *X. vasculorum*, *X. vesicatoria* and variety *raphani*, *X. hederae*, *X. papavericola*, *X. incanae*, *X. campestris* var. *amoraceae*, and *X. taraxaci*.

    III. The *phaseoli* group comprising *X. phaseoli* and its variety *fuscans*, *X. geranii*, *X. pelargonii*, and *X. malvacearum*.

    IV. The *translucens* group comprising *X. translucens* and its races, *X. begoniae*, *X. cucurbitae*, *X. juglandis*, *X. carotae*, and *X. maculafolium-gardeniae*.

    V. The *pruni* group comprising *X. pruni*, *X. corylina*, *X. phaseoli* var. *sojensis*, and *X. lespidizae*.

Six of the species or subspecies could not be placed in any group. The authors regard three of these (*X. hyacinthi*, *X. rubrilineans*, and *X. manihotis*) as doubtful xanthomonads. The remaining three *X. gummisudans*, *X. vignicola*, and *X. holciola*) did not show any serological affinity with the other organisms under study.

As a result of their researches Elrod and Braun (6) suggest a reduction in the number of species within the genus.

During the period 1934–36 the writer (17) worked on the taxonomy of the ''campestris'' group of the genus *Phytomonas* as defined by Burkholder (1). This is the present genus *Xanthomonas*. Cross inoculation studies among 16 taxonomically distinct hosts was a phase of the investigation. Inasmuch as 17 members inclusive of 14 species and 3 subspecies and representing 37 isolates (Table 1) are common to the investigations of both Elrod and Braun and the writer, the results of the writer have a distinct bearing on the problem; and although the results have been referred to in the literature (2, p. 134), it seems advisable to present them in greater detail in order to compare cross agglutination reactions with pathogenicity data for the purpose of determining species within the genus.

MATERIALS AND METHODS

Isolates were contributed by many people. Five were isolated by the author. For convenience, these are grouped alphabetically by species; the

TABLE 1.—Results[a] of cross inoculation experiments with isolates of species of Xanthomonas.

Species of Xanthomonas	Serological Group[b]	Isolate[c]	Brassica oleracea var. capitata	Corylus avellana	Cucurbita maxima	Geranium sanguineum	Hedera helix	Juglans regia	Gossypium hirsutum	Papaver rhoeas	Phaseolus vulgaris	Glycine max	Prunus persica	Saccharum officinarum	Hordeum vulgare	Triticum aestivum	Lycopersicon esculentum	Matthiola incana
1. campestris	I	1	+	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
		27	+	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
		*37	–	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
		57	+	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
2. corylina	II	78	–	+	–	0	–	–	–	–	–	–	–	0	–	–	–	0
3. cucurbitae	IV	*41	–	0	+	0	–	0	–	–	–	–	–	–	–	–	–	0
		45	–	0	+	0	–	0	–	–	–	–	–	–	–	–	–	0
		46	–	0	+	0	–	0	–	–	–	–	–	–	–	–	–	0
4. geranii	III	80	–	0	–	?	–	0	–	–	–	–	–	0	–	–	–	0
		81	–	0	–	+	–	0	–	–	–	–	–	0	–	–	–	0
5. hederae	II	16	–	0	–	0	+	0	–	–	–	–	–	–	–	–	–	0
6. juglandis	IV	77	–	–	–	0	–	+	–	–	–	–	–	–	–	–	–	0
		79	–	–	–	0	–	+	–	–	–	–	–	0	–	–	–	0
		82	–	–	–	0	–	+	–	–	–	–	–	0	–	–	–	0
7. malvacearum	III	64	–	0	–	0	–	0	+	–	–	–	–	–	–	–	–	0
8. papavericola	II	47	–	0	–	0	–	0	–	+	–	–	–	–	–	–	–	0
		48	–	0	–	0	–	0	–	+	–	–	–	–	–	–	–	0
9. phaseoli	II	5	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
		6	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
		7a	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
10. phaseoli var. fuscans	II	8	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
		9	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
11. phaseoli var. sojensis	V	11	–	0	–	0	–	0	–	–	±	+	–	–	–	–	–	0
12. pruni	V	21	–	0	–	0	–	0	–	–	–	–	+	–	–	–	–	0
		22	–	0	–	0	–	0	–	–	–	–	+	–	–	–	–	0
		24	–	0	–	0	–	0	–	–	–	–	+	–	–	–	–	0
		59	–	0	–	0	–	0	–	–	–	–	+?	–	–	–	–	0
13. rubrilineans	VI	63	–	0	–	0	–	0	–	–	–	–	–	+	–	–	–	0
14. translucens	IV	61	–	0	–	0	–	0	–	–	–	–	–	–	+	–	–	0
15. translucens var. undulosum	IV	62	–	0	–	0	–	0	–	–	–	–	–	–	–	?	–	0
		83	–	0	–	0	–	0	–	–	–	–	–	0	–	+	–	0
		84	–	0	–	0	–	0	–	–	–	–	–	0	–	+	–	0
16. vascularum	II	12	–	0	–	0	–	0	–	–	–	–	–	+	–	–	–	0
		49	–	0	–	0	–	0	–	–	–	–	–	+	–	–	–	0
17. vesicatoria	II	13	–	0	–	0	–	0	–	–	–	–	–	–	–	–	+	0
		38	–	0	–	0	–	0	–	–	–	–	–	–	–	–	+	0
		*39	–	0	–	0	–	0	–	–	–	–	–	–	–	–	+	0

[a] + = positive
– = negative
± = both positive and negative results
0 = no data
? = doubtful at time of writing
[b] I–VI = the serological group number of Elrod and Braun (6).
[c] * = albino strain

serological grouping of Elrod and Braun (6) is indicated by roman numerals.

(i) *Xanthomonas campestris* (Pammel) Dowson. Group I.
    1. from cauliflower, Aug., 1929. Dr. W. H. Burkholder, Ithaca, New York.
    27. from broccoli, Aug., 1932. Wernham, Ithaca, New York.
    37. an albino strain received from Miss Mary K. Bryan, Washington, D. C. Sept., 1932.
    57. from cabbage, Sept., 1932. Wernham, Ithaca, New York.

(ii) *X. corylina*. Miller *et al.* Group II.
    78. from English filbert. Dr. P. W. Miller, Corvallis, Oregon. Received May 15, 1935.

(iii) *X. cucurbitae* (Bryan) Dowson. Group IV.
    41. an albino strain received from Miss Mary K. Bryan. Sept., 1932.
    45. from cucumber, April, 1933. Miss Mary K. Bryan.
    46. from cucumber, April, 1933. Miss Mary K. Bryan.

(iv) *X. geranii* (Burkholder) Dowson. Group III.
    80. from *Geranium sanguineum*, June 4, 1935. Dr. W. H. Burkholder.
    81. ditto.

(v) *X. hederae* (Arnaud) Dowson. Group II.
    16. from *Hedera helix* collected at Rennes, France, Feb., 1932. Dr. W. H. Burkholder.

(vi) *X. juglandis* (Pierce) Dowson. Group IV.
    77. from English walnut. Dr. P. W. Miller. Received May 15, 1935.
    79. from English walnut. 1933. Dr. P. A. Ark, Berkeley, California. Received May, 1935.
    82. from English walnut, collected at Ukiah, California by Mr. C. E. Scott. May 24, 1935. Wernham.

(vii) *X. malvacearum* (E.F.S.) Dowson. Group III.
    64. from cotton, 1931. Miss Mary K. Bryan. Received Nov., 1933.

(viii) *X. papavericola* (Bryan and McWhorter) Dowson. Group II.
    47. from Shirley poppy, 1932. Miss Mary K. Bryan. Received April, 1933.
    48. from Oriental poppy, 1931. Miss Mary K. Bryan. Received April, 1933.

(ix) *X. phaseoli* (E.F.S.) Dowson.   Group II.

   5. from bean pods collected in Switzerland, Oct., 1927. Dr. W.
      H. Burkholder.
   6. from bean seed collected at Clifton Springs, N. Y., 1923.
      Dr. W. H. Burkholder. April, 1930.
   7a. a reisolate of a culture obtained from bean seed by Miss H.
      Nussle, Ithaca, N. Y. Wernham. (Subsequent to inocula-
      tion the original culture was lost.)

(x) *X. phaseoli* var. *fuscans* (Burkholder) Starr and Burkholder.
      Group II.

   8. from bean pods collected in Switzerland, Dec., 1924. Dr. W.
      H. Burkholder.
   9. ditto. Oct., 1927.

(xi) *X. phaseoli* var. *sojensis* (Hedges) Starr and Burkholder.   Group
      V.

   11. from soybeans. Miss Florence Hedges, Washington, D. C.
      Sent to Dr. W. H. Burkholder, March, 1930.

(xii) *X. pruni* (E.F.S.) Dowson.   Group V.

   21. from prune, Aug. 16, 1932. Dr. W. H. Burkholder.
   22. ditto.
   23. isolated by Dr. H. H. Thornberry, Boyce Thompson Institute,
      Yonkers, N. Y. Received Aug., 1932.
   59. from wild cherry collected at Georgetown, N. Y., Aug. 28,
      1933. Wernham.

(xiii) *X. rubrilineans* (Lee *et al.*) Starr and Burkholder.   Group VI.

   63. received Nov., 1933, from Miss Florence Hedges through the
      courtesy of Miss Charlotte Elliott.

(xiv) *X. translucens* (Jones *et al.*) Dowson.   Group IV.

   61. from barley, September., 1933. Dr. C. S. Reddy, Ames, Iowa.

(xv) *X. translucens* var. *undulosum* (S. J. and R.) Hagborg.   Group
      IV.

   62. from wheat, Sept., 1933. Dr. C. S. Reddy.
   83. received from Dr. R. A. Bamberg, University Farm, St. Paul,
      Minnesota, March 27, 1936.
   84. ditto.

(xvi) *X. vascularum* (Cobb) Dowson.

   12. isolated in 1923 at Bureau of Plant Industry. Received by
    · Dr. W. H. Burkholder, March, 1930.

serological grouping of Elrod and Braun (6) is indicated by roman numerals.

(i) *Xanthomonas campestris* (Pammel) Dowson. Group I.
  1. from cauliflower, Aug., 1929. Dr. W. H. Burkholder, Ithaca, New York.
  27. from broccoli, Aug., 1932. Wernham, Ithaca, New York.
  37. an albino strain received from Miss Mary K. Bryan, Washington, D. C. Sept., 1932.
  57. from cabbage, Sept., 1932. Wernham, Ithaca, New York.

(ii) *X. corylina.* Miller *et al.* Group II.
  78. from English filbert. Dr. P. W. Miller, Corvallis, Oregon. Received May 15, 1935.

(iii) *X. cucurbitae* (Bryan) Dowson. Group IV.
  41. an albino strain received from Miss Mary K. Bryan. Sept., 1932.
  45. from cucumber, April, 1933. Miss Mary K. Bryan.
  46. from cucumber, April, 1933. Miss Mary K. Bryan.

(iv) *X. geranii* (Burkholder) Dowson. Group III.
  80. from *Geranium sanguineum,* June 4, 1935. Dr. W. H. Burkholder.
  81. ditto.

(v) *X. hederae* (Arnaud) Dowson. Group II.
  16. from *Hedera helix* collected at Rennes, France, Feb., 1932. Dr. W. H. Burkholder.

(vi) *X. juglandis* (Pierce) Dowson. Group IV.
  77. from English walnut. Dr. P. W. Miller. Received May 15, 1935.
  79. from English walnut. 1933. Dr. P. A. Ark, Berkeley, California. Received May, 1935.
  82. from English walnut, collected at Ukiah, California by Mr. C. E. Scott. May 24, 1935. Wernham.

(vii) *X. malvacearum* (E.F.S.) Dowson. Group III.
  64. from cotton, 1931. Miss Mary K. Bryan. Received Nov., 1933.

(viii) *X. papavericola* (Bryan and McWhorter) Dowson. Group II.
  47. from Shirley poppy, 1932. Miss Mary K. Bryan. Received April, 1933.
  48. from Oriental poppy, 1931. Miss Mary K. Bryan. Received April, 1933.

(ix) *X. phaseoli* (E.F.S.) Dowson.   Group II.

    5. from bean pods collected in Switzerland, Oct., 1927. Dr. W. H. Burkholder.

    6. from bean seed collected at Clifton Springs, N. Y., 1923. Dr. W. H. Burkholder. April, 1930.

    7a. a reisolate of a culture obtained from bean seed by Miss H. Nussle, Ithaca, N. Y. Wernham.   (Subsequent to inoculation the original culture was lost.)

(x) *X. phasₒoli* var. *fuscans* (Burkholder) Starr and Burkholder. Group II.

    8. from bean pods collected in Switzerland, Dec., 1924. Dr. W. H. Burkholder.

    9. ditto. Oct., 1927.

(xi) *X. phaseoli* var. *sojensis* (Hedges) Starr and Burkholder. Group V.

    11. from soybeans. Miss Florence Hedges, Washington, D. C. Sent to Dr. W. H. Burkholder, March, 1930.

(xii) *X. pruni* (E.F.S.) Dowson. Group V.

    21. from prune, Aug. 16, 1932. Dr. W. H. Burkholder.

    22. ditto.

    23. isolated by Dr. H. H. Thornberry, Boyce Thompson Institute, Yonkers, N. Y. Received Aug., 1932.

    59. from wild cherry collected at Georgetown, N. Y., Aug. 28, 1933. Wernham.

(xiii) *X. rubrilineans* (Lee *et al.*) Starr and Burkholder. Group VI.

    63. received Nov., 1933, from Miss Florence Hedges through the courtesy of Miss Charlotte Elliott.

(xiv) *X. translucens* (Jones *et al.*) Dowson. Group IV.

    61. from barley, September., 1933. Dr. C. S. Reddy, Ames, Iowa.

(xv) *X. translucens* var. *undulosum* (S. J. and R.) Hagborg. Group IV.

    62. from wheat, Sept., 1933. Dr. C. S. Reddy.

    83. received from Dr. R. A. Bamberg, University Farm, St. Paul, Minnesota, March 27, 1936.

    84. ditto.

(xvi) *X. vascularum* (Cobb) Dowson.

    12. isolated in 1923 at Bureau of Plant Industry. Received by Dr. W. H. Burkholder, March, 1930.

49. from sugar cane. Dr. Mel. T. Cooke, Rio Piedras, Porto Rico. Received May, 1933.

(xvii) *X. vesicatoria* (Doidge) Dowson. Group II.
13. from tomato fruits, Aug., 1929. Dr. W. H. Burkholder.
38. received from Miss Mary K. Bryan, Sept., 1932.
39. an albino strain received from Miss Mary K. Bryan, Sept., 1932.

All bacteria used were tested for pathogenicity on their original suscept, and those that were non-virulent were discarded.

Most of the plants used in the inoculation experiments are the hosts on which the pathogens were originally described. Of these plants the following were grown from seeds: cabbage, cotton, Shirley poppy, tomato, soybean, common bean, squash, barley, and wheat. Potted plants of English ivy and stocks were used. Cuttings of sugar cane were received from Louisiana. Six plants each of English walnut and English filbert were purchased from a commercial firm. June buds of Elberta peaches were furnished by a nursery. Only one plant of *Geranium sanguineum* was available.

Three methods were used in inoculating plants. Transfers from stock cultures were made to Difco nutrient agar slants and incubated for 48 hours at 27° C. The bacteria were (a) smeared over the leaf or applied in water suspension, and pricked through by means of a sheaf of sterile insect needles or (b) scraped off the slant on the tip of a sterile scalpel and introduced into the vascular system on the scalpel point or (c) atomized or brushed onto leaves by means of sterile atomizers or camel's hair brushes.

After inoculation species of small plants were placed in an incubation chamber of 85–95 per cent relative humidity for 48 hours. Wherever possible two series of plants were used, each series at a different time in the investigation. Each series allowed at least one plant; individual leaves made possible many single inoculations. The material of sugar cane, walnuts, and filberts was too scarce for more than pathogenicity tests. Cultures added to the study after scarce material was discarded were not tested. Of all the cross inoculations made, only the results on sugar cane are subject to misinterpretation. With this species symptoms were confusing.

Infection was considered positive only when the bacteria were able to establish themselves and cause a progressive disease. Thus injury due to needle pricks plus toxic material of the inoculum was considered negative.

CROSS INOCULATIONS

A study of the cross inoculation results shows this group of pathogens to be remarkably specific in their host reactions (Table 1). This is in marked contrast to the results reported by Clara (3) in his study of the green fluorescent plant pathogens. Some yellow isolates which were re-

ceived as authentic cultures proved to be non-pathogenic in spite of repeated attempts to rejuvenate them, and they are not listed here. Of the three albino strains included in the study, two were pathogenic, showing that a color mutation is not necessarily accompanied by loss of virulence.

Pathogenicity as a species concept is definitely of value in the species studied. With the single exception of the subspecies *phaseoli fuscans* which can be distinguished readily from *phaseoli* by the soluble brown pigment it imparts to the agar, there is no possibility of error in using the host as a species determiner.

Both *Xanthomonas vascularum* and *X. rubrilineans* infect sugar cane but produce different symptoms. Furthermore, while the former is a true species of *Xanthomonas,* the latter shows few cultural characteristics of the genus according to Starr and Burkholder (14). Serologically Elrod and Braun also found no relationships.

The five serological groups of Elrod and Braun show no correlation with the pathogenicity of the various species investigated in this article. Furthermore, the species of this genus that hydrolyse or do not hydrolyse starch, the few species that are not lipolytic (14), the one species that breaks down tyrosine into a melanin (*X. phaseoli* var. *fuscans*) and the "fastidious" species of Starr (13) while they help in defining the phytogenic species do not correlate with the serological grouping. Although it is of interest to know the serological relationships in the genus, the plant pathologist will insist on a nomenclature to differentiate the phytopathogenic species.

DISCUSSION

In order to place the data presented above in proper perspective it appears to be necessary to present the cogent reasons for the serological study of Elrod and Braun. These authors (6, p. 509) state:

"Since establishment of the genus, the more than 40 members have assumed a more rational place in bacteriological taxonomy but are still little understood as a group. It is unfortunate that these organisms . . . , have received little study. We have scant knowledge of the relationships between the various species and subspecies. Inasmuch as it is the practice of the phytopathologist in general to form a new species if a bacterial plant pathogen of a recognized genus is isolated from a new host, it is apparent that many species could have been duplicated. This practice of basing species differentiation almost solely on host of isolation (with the host range almost always inadequately investigated) has resulted in a seeming increase in the size of the genus, giving rise to the belief among certain workers that all the members may not represent true species.

As an approach to this problem we have undertaken an extensive serological survey of the group to determine whether the genus could be logically classified in this fashion and to ascertain whether antigenic characteristics could be correlated with host range."

Examination of the references cited by these authors reveals that the

literature (4, 8, 9, 13, 14, 16) had been covered with the possible exception of Burkholder's article of 1939 (2). It is difficult indeed to interpret the polemical approach and its reiteration throughout the discussion, had the authors been aware of this paper. Nevertheless the data are established and it is necessary to evaluate them in the light of cross inoculation results presented above and that previously published by others.

The serological relationships do not correlate with the host range as determined by the author, nor do the results of Wallin (16) coincide with the groupings of the *translucens* isolates in which we find *X. translucens* f. sp. *hordei* in both the "cucurbitae" and the "cerealis" subgroups. These data are also at variance with the serological findings of Hagborg (9). A more recent paper (7) by Elrod and Braun emphasizes these discrepancies. In this publication (7, p. 356) the authors state: "It is felt that the antigenic properties of an organism vary independently with the ability to infect given host species."

These findings and statements are in general agreement with those of Stevens (15) who carried on similar investigations with the legume nodule-forming bacteria. Seven species of these bacteria, based on host plant infection, were studied. Agglutination differentiated the 55 cultures into 18 serological groups. Stevens' results indicated "the impossibility of identifying an organism by means of the agglutination test."

The writer believes his data, together with those of Hagborg (8), Wallin (16), and earlier workers (10) are sufficiently ample to show clearly that serological studies, regardless of their potential usefulness, are subordinate in value to pathogenicity as species determiners in the genus *Xanthomonas* at the present level of our information.

The writer believes, in addition, that any technique of species identification that is beyond the immediate faculties of the research worker primarily concerned with the species, fails as an implement of usage in any system of classification.

SUMMARY

1. Cross inoculation studies with 17 members of the genus *Xanthomonas* on 16 taxonomically distinct hosts reveal pathogenicity to be a remarkably specific characteristic of these species.

2. There is an almost complete lack of correlation between pathogenicity and the serological grouping of the species as reported by Elrod and Braun (6).

3. The data indicate that, within the genus, pathogenicity is of primary consideration as a species determiner, in the light of our present knowledge of the group.

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A TOXIC METABOLIC PRODUCT OF FUSARIUM OXYSPORUM VAR. NICOTIANAE IN RELATION TO A WILTING OF TOBACCO PLANTS[1]

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Among the theories attempting to explain the wilting of plants infected with vascular parasites of the genus *Fusarium,* the toxin theory has been supported by most investigations within recent years and has gained most wide acceptance. This theory is based primarily upon observations that these fungi, when grown upon artificial media, produce toxins capable of wilting healthy plants. Organisms for which the production of wilt-inducing toxins have been demonstrated include *Fusarium cubense,* causing the Panama disease of banana (2, 16), *F. solani* (5, 21), *F. oxysporum* from potato (1, 9, 13, 28), the cotton wilt organism, *F. vasinfectum* (18, 20, 21, 24, 25), the pea wilt organism, *F. orthoceras* var. *pisi* (17), *F. lini,* causing flax wilt (12, 19), and the tomato wilt organism, *F. lycopersici* (or *F. bulbigenum* var. *lycopersici*) (3, 6–8, 10, 11, 14, 19, 22, 23, 26, 27). The literature on the wilting problem has been recently summarized by Gottlieb (11) and hence will not be considered in detail.

The toxin theory has been strengthened by the findings of Wellman (26), who reported differences in the toxicity of culture filtrates of mild and virulent strains of *Fusarium lycopersici,* increased toxin production being associated with greater virulence. In *F. lycopersici,* Gottlieb (10) has provided direct evidence in support of the toxin theory by the demonstration of toxins within the tracheal systems of wilted plants, this constituting the only instance for any Fusarium disease in which a toxin has been recovered from an infected host plant.

Efforts to identify the chemical compounds concerned in wilting have been successful in relatively few cases. The toxic metabolic product of various Fusaria has been identified as an aldehyde (16), nitrite (24), an amine (12, 18, 25), ammonia (4, 19), and an alcohol (19). Recent studies by a group of Swiss investigators have resulted in the isolation and characterization of a polypeptide-like toxin from filtrates of *Fusarium lycopersici* (3, 22, 23). The culture filtrate was treated with $Ba(OH)_2$ to remove sulphate and phosphate ions, and evaporated to dryness *in vacuo*. The residue was then dissolved in water and the toxic material was precipitated with methanol. Additional procedures resulted in the isolation of a pure crystalline substance, lycomarasmin, having the empirical formula $C_9H_{15}O_7N_3$ (22). Upon hydrolysis, lycomarasmin was found to yield glycine, aspartic acid,

ammonia, and probably pyruvic acid (23). Other studies by this group in-
volve the physiological action of this toxin, which in exceedingly small con-
centrations causes wilting of tomato leaves (3, 7, 8). These workers also
sought to explain the nature of the mechanisms concerned in wilting and the
relation of transpiration to the wilting process.

The present study is concerned with *Fusarium oxysporum* var. *nicoti-
anae*, originally described by Johnson (15) as the cause of a wilt disease of
tobacco. A proximate cause of wilting was sought by determining whether
toxic metabolic products are elaborated by this organism, and, since this
proved to be the case, efforts were made to learn something of the properties
of the toxin.

MATERIALS AND METHODS

A culture of *Fusarium oxysporum* var. *nicotianae* was obtained from Dr.
G. B. Lucas of the Tobacco Experiment Station, Oxford, N. C. The fungus
was grown on a modified Richards' solution having the following composi-
tion: KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, trace; glucose,
34 gm.; and distilled water, to make 1000 ml. Cultures were maintained
at room temperature in 1-liter Erlenmeyer flasks containing 200 ml. of
medium.

Young tobacco plants with 6–8 well-developed leaves were employed to
assay the culture filtrates. The plants were grown in plant bands and when
ready for use the roots were washed free of adhering soil particles, and the
plants were then placed in 10-ml. vials with their roots submerged in the
solutions to be tested.

Inasmuch as this study was preliminary in nature, undertaken to estab-
lish the possible existence of a wilt-inducing toxin in the fungal filtrates and
to learn some of the chemical properties of the toxic material, environmental
conditions under which the tests were made were not controlled, and the
results are therefore qualitative or semiquantitative in character.

RESULTS

Fusarium oxysporum var. *nicotianae* grows well on Richards' solution,
the mycelium forming a pink mat over the surface of the medium in about
10 days. The medium gradually becomes discolored owing to the secretion
of metabolic products by the fungus.

Preliminary tests of the wilting power of filtrates from cultures of
different ages disclosed that a growth period of at least one month is required
before the filtrate has attained maximum toxicity. All subsequent experi-
ments were performed using filtrates of cultures approximately one month
old.

From 18 to 48 hours are required for the production of symptoms of
toxicity in plants placed in the filtrates. Development of symptoms begins
with the lowermost leaf, and progresses upward. The symptoms include a
drooping and wilting of the leaves and stem tip, multiple small areas of
necrosis in the laminar tissues, and collapse of vascular elements in the

proximal portions of the leaves. When wilted plants in incipient stages were placed in distilled water they failed to recover, indicating that such wilting is irreversible in character and must be regarded as pathological wilting.

Control plants placed in noninoculated Richards' solution were observed to become flaccid, a result in agreement with the findings of a number of previous investigators. Such plants recovered spontaneously within a few hours, however, and invariably recovered when transferred to distilled water. Such temporary wilting obviously is due to osmotic phenomena and must be differentiated from pathological wilting occurring when fungus toxins are present.

The filtrate was found capable of producing wilt and other associated symptoms when diluted 1:10, but not in greater dilution. When the filtrate was evaporated to dryness at 100° C., and the residue was redissolved in distilled water to restore the original volume, there was no apparent loss of toxicity. It may be concluded, therefore, that the toxic material is thermostable.

When the mycelial mat was macerated by grinding with quartz sand in a mortar and extracted in distilled water, the extract induced wilting of test plants. Further study was not made, however, of toxic substances within the mycelium.

When the filtrate was treated with an equal volume of $0.2\,N\,Ba(OH)_2$, a copious precipitate containing sulphate, phosphate, and presumably the salts of organic acids was formed. Because of its high salt content, and consequent possibility of causing wilting through osmotic action, this precipitate was not tested for toxic activity. The filtrate from the $Ba(OH)_2$ treatment, however, was evaporated to dryness *in vacuo* at 50°-60° C. Portions of the distillate were collected, and when tested proved completely non-toxic. The residue, when redissolved in water, appeared to be as toxic to test plants as the original filtrate. The toxic material is therefore non-volatile.

The pH of the culture solution, originally 4.6, became increasingly more alkaline, reaching 7.8 after growth of the organism for one month. Adjustment of the filtrate to pH 4.6 did not affect the production of toxic symptoms and indicates that alkalinity itself is not the cause of wilting.

After growth of *Fusarium oxysporum* var. *nicotianae* for one month, the filtrate gave no reaction with Fehling's solution or Benedict's solution, indicating that the glucose originally present had completely disappeared. An aldehyde does not seem responsible for the toxicity, and a test for ketones with Girard's reagent was likewise negative.

A test of the filtrate for nitrate with Trommsdorf's reagent was negative. A test for nitrite with sulphanilic acid and alpha-naphthylamine was also negative, indicating that nitrite, demonstrated by Rosen (24) to be the toxic agent in filtrates of *Fusarium vasinfectum,* is not responsible for the toxicity of filtrates of *F. oxysporum* var. *nicotianae.*

The filtrate gave a positive ninhydrin reaction with triketohydrindene hydrate, and a very strongly positive reaction for ammonium with Nessler's reagent. In view of the claims of toxicity of ammonium by Luz (19) and Elpidina (4), experiments were made to determine whether ammonium ions were responsible for toxicity of filtrates of *Fusarium oxysporum* var. *nicotianae*. Attempts to perform tests with filtrates treated with Nessler's reagent to remove ammonium failed, owing to toxicity of the Nessler's reagent itself, which induced severe vascular collapse in test plants. After the filtrate, which was alkaline in reaction, had been evaporated to dryness at 100° C., and the original volume had been restored with distilled water, a portion of it was tested with Nessler's reagent and was found to be negative, indicating that the ammonia had been completely volatilized. Another portion of this solution was found to have retained its toxic properties after removal of ammonia.

Since ammonium was not the toxic constituent, it was thought desirable to test for amines, in view of the results of Schaffnit and Lüdtke (25) and Lüdtke and Achmed (18). Determination of amino nitrogen in the filtrate, by the method of Van Slyke, gave the low value of 0.04 per cent. Further, the residue from the culture filtrate, when evaporated to dryness *in vacuo*, dissolved in a minimal quantity of distilled water, and tested with benzoyl chloride, failed to give the reaction characteristic of primary and secondary amines.

The possible protein nature of the toxic material was tested for the reason that certain well known bacterial toxins are known to be proteinaceous. The filtrate gave negative reactions with Millon's reagent and the xanthoproteic test, however. Further, an attempt to precipitate proteins from the filtrate by the addition of $(NH_4)_2SO_4$ equivalent to 4.2 M (including a correction for solution volume) gave no precipitate in 24 hours at a maintained temperature slightly above 0° C.

In order to test for the presence of lycomarasmin, the procedure of Plattner and Clauson-Kaas (22) was followed: After treatment of the filtrate with an equal volume of $0.2 N Ba(OH)_2$ and filtration, the resulting filtrate was evaporated to dryness *in vacuo* at 50°–60° C., and the residue dissolved in the minimal amount of distilled water. The solution was then treated with charcoal, filtered, made acid to litmus, cooled in an ice bath to 0° C., and 3 volumes of absolute methyl alcohol were added. The material was then allowed to remain in a cold room for 24 hours after which the white flocculent precipitate was removed by filtration. When tested upon plants this precipitate was completely inactive. The aqueous-methyl alcohol filtrate was reduced in volume by vacuum distillation and shown to contain the toxic factor. The toxin of *Fusarium oxysporum* var. *nicotianae* is therefore not lycomarasmin nor any closely related polypeptide.

Three residues were prepared as follows: Culture filtrate, untreated, was evaporated to dryness *in vacuo*. To a second portion of culture filtrate was added an equal volume of $0.2 N Ba(OH)_2$ to remove sulphate and phos-

phate, and after filtration the filtrate was evaporated to dryness *in vacuo*. Similarly, after precipitation with $0.2 N Ba(OH)_2$, a third portion of filtrate was neutralized and then evaporated to dryness *in vacuo*. No apparent difference in the toxicity of these three residues was noted. It may be observed, however, that the alkaline filtrate yielded a hygroscopic dark brown residue, whereas the residue obtained after the addition of $Ba(OH)_2$ and neutralization was crystalline and possessed only a small amount of color.

Efforts were made to extract the toxic principle from the mixture of substances present in these residues through the use of various solvents. In these experiments the residue was repeatedly extracted with successive small volumes of the chosen solvent, the solvent was then removed by evaporation *in vacuo* and the soluble and insoluble portions were dissolved in distilled water and tested upon plants.

It was found that the toxin is insoluble in ether, and cannot be extracted by this solvent from the residue or from the original culture filtrate. Similarly, the toxic principle is insoluble in acetone. Toxic substances could be extracted from the residue, however, with methyl alcohol or with ethyl alcohol. With methyl alcohol, separation of a factor that induces wilting from one that induces necrotic symptoms could be obtained. The methyl alcohol-soluble portion produced severe wilting not accompanied by necrosis, while the fraction insoluble in methyl alcohol produced marked necrosis but only moderate wilting. This would appear to indicate that at least two toxic substances are metabolized by *Fusarium oxysporum* var. *nicotianae*.

DISCUSSION

The production of thermostable, nonvolatile substances toxic to plants appears to be a rather common characteristic of the pathogenic species of *Fusarium*. Because Richards' solution has so frequently been the medium chosen to demonstrate the elaboration of toxic materials by Fusaria, one wonders whether results obtained with cultures on this medium are really applicable to Fusarium diseases as they occur in plants in the field, and whether toxins are elaborated when the Fusaria are invading the vascular tissues. The demonstration of a toxin within the tissues of an infected plant (10) constitutes convincing evidence bearing on this phase of the problem, and indicates the need for similar studies with other species of *Fusarium*. The failure of Rosen (24) to obtain toxin from *F. vasinfectum* when grown in Uschinsky's solution or in nutrient broth indicated that the formation of toxins is conditioned by the composition of the medium. The need for studies of the effects of various carbon and nitrogen sources, minerals, and accessory growth factors upon toxin formation is apparent.

Another point, which has been noted by almost all workers, but to which apparently far too little significance has been attached, is that a prolonged period of growth, of the order of 4–6 weeks, is required for the formation of maximal amounts of toxin in cultures of Fusaria. Luz (19) states that the

substances most active in the induction of wilting appear to be related to the death of the mycelium. With the single exception of lycomarasmin, the toxins of Fusaria are not active in great dilution. One must infer, therefore, that they are produced in very small amounts, and must act over considerable periods in order to induce wilting in nature. In support of this conclusion, it may be recalled that tobacco plants, presumably invaded soon after transplanting in the field, may become almost mature before showing symptoms. In this disease, and presumably in other Fusarium diseases as well, the disease process must be characterized as chronic rather than acute.

All too little is known of the chemical nature of the toxins produced by Fusaria. The present results, admittedly inadequate, appear to indicate considerable similarity in the properties of the toxic materials produced by *Fusarium oxysporum* var. *nicotianae* with those produced by *F. vasinfectum* according to the results of Lüdtke and Achmed (18), and by *F. lini*, according to the work of Grossmann (12).

SUMMARY

When cultivated in a modified Richards' solution, *Fusarium oxysporum* var. *nicotianae* produces material which causes wilting and other symptoms of toxicity in tobacco plants. Both the culture filtrate and the fungus mycelium have toxic properties.

The chemistry of the toxic principle remains incompletely known. The toxin is thermostable and nonvolatile. Evidence is presented indicating that it is insoluble in ether or acetone, partially soluble in methyl alcohol and ethyl alcohol, and that it is not a nitrite, an ammonium salt, an aldehyde, a ketone, an amine, or a protein, and is not identical with lycomarasmin. More than one toxic factor appears to be involved, one of which is primarily concerned with wilting and the other with necrosis.

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QUANTITATIVE DETERMINATION OF TETRACHLORO-p-BENZOQUINONE ON TREATED SEED

H. P. BURCHFIELD AND G. L. McNEW [1]

Simple, reliable methods for measuring the dosage of fungicidal protectants on seeds are needed both by scientists and practical agriculturists. The addition of a prescribed weight of chemical does not assure the desired dosage since much of the material may be lost on the treating equipment, by dispersion into the air, and by dislodgement in handling. Experience has shown that even the laboratory investigator who has rigid control over his methods frequently fails to secure complete, uniform coverage. An analysis of the treated seed for fungicide content would, therefore, contribute materially to the reliability of both the experimental and practical use of chemicals.

Since tetrachloro-para-benzoquinone (Spergon) has been recommended for use on pea (5, 6, 9, 10, 12), lima bean (1, 8), corn (5, 7, 9, 11) and other seed (2, 13) during the past six years, a quantitative method of analysis was considered desirable. This compound reacts with basic reagents to form highly colored substitution products. An effort was made to take advantage of this in developing a colorimetric method for the quantitative determination of tetrachlorobenzoquinone. The results of these studies and a new method of analysis are described in this paper. Data are presented on the reliability of the method when applied to treated seed of maize, peas, lima beans, beet, and spinach.

DESCRIPTION OF CHEMICAL PROCESSES

The best known reaction of tetrachlorobenzoquinone with basic materials is with aqueous sodium hydroxide to form the red-violet sodium chloranilate
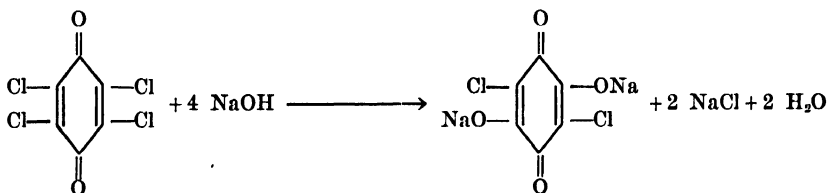
(3). Although the color is intense, the reaction is not satisfactory for use on treated seed. The hydrolysis of tetrachlorobenzoquinone is slow, and the caustic frequently removes natural pigments, particularly from maize seed. In order to avoid these difficulties it was necessary to first remove the tetrachlorobenzoquinone from the seed by use of a selective solvent. It

was found that three or four washings in acetone dissolved all of the material.

A method was developed, and used for some time, in which the acetone solution from 5 gm. of seed was made up to about 130 ml., treated with 50 ml. of 0.05 N sodium hydroxide, made to 250-ml. volume with acetone, and the optical density measured with a colorimeter. An intense yellow color develops which is proportional to the concentration of tetrachlorobenzoquinone. It is reasonable to assume that this yellow compound is an intermediate acetone condensation product since tetrachlorobenzoquinone is known to react with compounds such as malonic ester (14) and acetoacetic ester (4), that contain labile hydrogen:

$$\text{Cl---}\underset{O}{\overset{O}{\bigcirc}}\text{---Cl} + 2\ RH + 2\ NaOH \longrightarrow \text{Cl---}\underset{O}{\overset{O}{\bigcirc}}\text{---R} + 2\ NaCl + 2\ H_2O$$
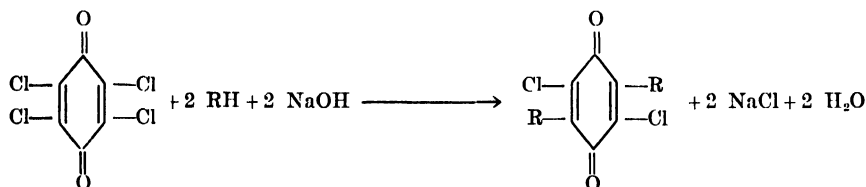
This method is not satisfactory for general use because the yellow color is unstable and varies in intensity with slight differences in the quality of the acetone.

It was assumed that the fading was caused by hydrolysis; so studies were undertaken to eliminate water from the reaction. This was accomplished by substituting anhydrous diethylamine for the sodium hydroxide solution. The addition of this reagent immediately produced a yellow-green coloration that changed rapidly to an intense yellow. No further appreciable change in optical properties occurred during the next 15 minutes. The addition of a trace of water, however, caused rapid fading; so both the reagents and glassware must be free of moisture. Measurements made with a Beckman spectrophotometer showed that the yellow reaction product had an absorption maximum at about 450 mμ (Fig. 1).

Complete proof is not available on the nature of the reaction between tetrachlorobenzoquinone, acetone and diethylamine. The absorption spectrum does not agree with that of 2.,5-bis (diethylamino)-3,6-dichloro-p-benzoquinone, the normally anticipated reaction product. It closely resembles the acetone condensation product described above and probably is identical to it. Regardless of the mechanism involved, the reaction seemed suitable for photometric analysis and was adapted to the determination of tetrachlorobenzoquinone.

<center>MATERIALS AND METHODS</center>

In order to determine the reliability of the acetone-diethylamine reaction for assaying tetrachlorobenzoquinone, a highly refined sample (M.P. 290°C), purified by chromatographic adsorption of the impurities on talc, was tested at different concentrations. Aliquots of 25, 20, 10, 5, and 2 ml. were transferred from a stock solution containing 0.25 gm. per liter to

50-ml. volumetric flasks and made up to about 40 ml. with acetone. Five ml. of anhydrous diethylamine was added and the mixtures made to volume with acetone. After standing for 3 min., the samples were placed in a Lumetron colorimeter equipped with matched 1-cm. absorption cells and
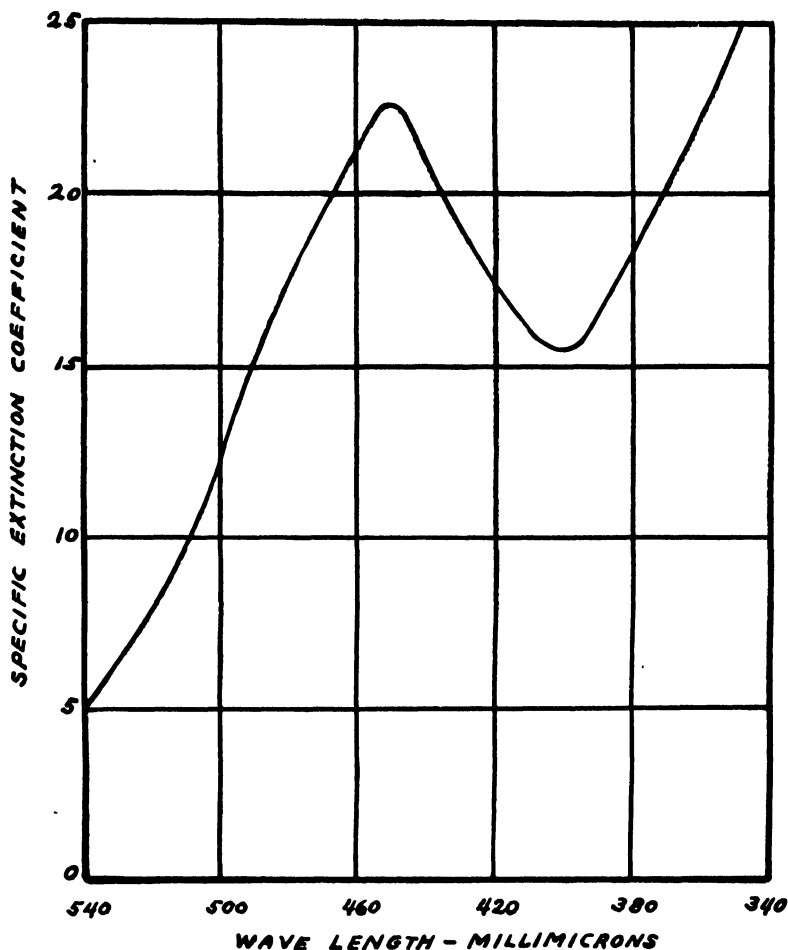
FIG. 1. Absorption spectrum of the reaction product of tetrachloro-p-benzoquinone with diethylamine and acetone.

a blue filter. The percentages of light transmitted (T) were measured against an acetone blank, converted to optical densities (log 100/T) and plotted against the concentration of tetrachlorobenzoquinone. A linear relationship was found that demonstrates adherence to the Beer-Lambert law. By application of the following formula, the apparent specific extinction coefficient (K) was calculated to be 17.3.

$$K = \frac{\log \frac{100}{T}}{G}$$

where G equals the concentration of tetrachlorobenzoquinone in grams per liter in the final solution on which readings were taken.

The application of this method to treated seed was relatively simple. The amount of seed and the dilutions required were found to vary with the type of seed and dosage of fungicide. Preliminary tests indicated that the proportions outlined in table 1 were the most satisfactory. Large

TABLE 1.—*Preferred quantity of seed and dilutions used in colorimetric analysis*

Kind of seed to be analyzed	Minimum amount of seed required	Initial volume of solution	Size aliquot for analysis of seed bearing a dosage of				
			0.50 per cent	0.25 per cent	0.12 per cent[a]	0.06 per cent	0.03 per cent
	gm.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
Lima bean	25.0	250	2	5	10	25	25
Corn	5.0	50	2	5	10	25	25
Peas	5.0	50	2	5	10	25	25
Spinach	2.5	25	2	5	10	25	25
Beet	2.5	25	2	5	10	25	25

[a] Approximately one ounce per bushel of pea or corn seed.

smooth seed such as those of lima beans gave irregular results unless a relatively large sample was used; while small, rough seeds such as those of spinach gave consistent results with samples as small as 2.5 grams.

The following procedures were employed in determining the concentration of chemical on the treated seed. The seed were weighed to the nearest 0.01 gm. on a torsion balance. The tetrachlorobenzoquinone was dissolved by three successive washings in acetone (analytical reagent grade) and the combined extracts made to the volume recommended in table 1 for seed samples of different sizes. The solution was then filtered through a fluted No. 5 Whatman paper to remove dust and seed fragments. An aliquot whose size depended on the anticipated dosage of tetrachlorobenzoquinone (Table 1) was transferred to a volumetric flask and made up to about 40 ml. with acetone. Five ml. of diethylamine was added, the mixture made to volume with acetone and after 3 min. was placed in the colorimeter. The percentage transmission was measured against an acetone blank.

The dosage of tetrachlorobenzoquinone on the seed was calculated from the following equation in which Vo is the initial volume of solution, V the volume of the aliquot, T the percentage of light transmitted, W the weight of the seed, and K the apparent specific extinction coefficient of the reaction product.

$$\text{Percentage TCPB} = \frac{5 \, Vo \log \frac{100}{T}}{KVW}$$

The applicability of the foregoing method was evaluated extensively on seed of hybrid dent corn and peas of the variety Perfection. Samples of 250 gm. of corn and 200 gm. of peas were weighed out to the nearest

0.01 gm. on a torsion balance (120-gm. capacity). These samples were placed in quart jars and sufficient chemical added to provide for dosages of 0.48, 0.24, 0.12, 0.06, and 0.03 per cent by weight after a nominal loss on the walls of the jar. The jars were then rotated on a vertical turntable for 30 min. at 28 r.p.m. to distribute the chemical uniformly on the seed. The samples were reweighed to determine the amount of fungicide adhering to them. Correction was made for any loss or gain in the untreated control, presumably due to moisture loss or uptake from the air.

The weighing of 200 grams or more of seed to detect 0.06 to 0.96 gm. of chemical is not so accurate as would be desired, since an error of 0.01 gm. may appear as 1 to 16 per cent error in dosage, depending upon the dosage level under investigation. Three different commercial preparations (Spergon, about 98 per cent active; Spergon-DDT-S1, about 96 per cent active; and wettable Spergon, about 48 per cent active) were applied at each dosage level to both types of seed.

Since discrepancies were anticipated in the data on dosages obtained by weighing and analysis, a third, independent measurement of dosage was sought as a guide and referee. Since the decay of seed in soils infested with *Pythium* sp. and *Rhizoctonia* sp. is directly affected by the dosage of chemical (9), 16 replications of 25 seeds each were sown in randomized block arrangement in greenhouse flats filled with naturally infested composted soil. The seed was covered to a uniform depth, watered heavily, placed in a cabinet at 55° F. for seven days and then removed to a greenhouse at 65° to 80° F. Records on emergence were taken 10 days later and subjected to analyses of variance.

EXPERIMENTAL RESULTS

Data obtained on the dosage of fungicide on the various samples of corn and pea seed by analytical and weighing methods are presented in table 2. The results by the two methods are in strikingly close agreement. Divergence at the highest dosage ranged from 0 to 8 per cent while at the lowest dosage the discrepancies ranged from 0 to 30 per cent of the total chemical. This latter figure is little more than would be expected from the error inherent in the weighing method.

The response of seed treated with different dosages to decay fungi was very uniform within each series of chemicals and between chemicals when allowance is made for differences in the percentages of active ingredient. All the analytical data have been corrected to account for the inert diluents and conditioning agents present in the formulations.

The analytical data in table 2 are the means of three separate determinations on 5-gm. samples of seed. The average range of these triplicate tests was 5.64 per cent of the total chemical present. The standard deviation for single determinations was 3.3 per cent of the chemical. This compares to a standard deviation of 1.3 per cent for the analytical constant obtained on a stock solution of the pure chemical. It follows that the

errors due to variations in extraction and distribution of chemical on the seed must be in the order of 3.05 per cent. Furthermore, it is obvious that a method embodying such precision would not lead to discrepancies

TABLE 2.—*Comparative dosage of Spergon on corn and pea seed determined by weighing and colorimetric analysis*

Treatment applied		Dosage on corn[a]			Dosage on peas[a]		
Material used	Desired dosage	By weight	By analysis	Emergence of corn	By weight	By analysis	Emergence of peas
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Spergon	0.48	0.46	0.461	86.8	0.45	0.455	91.0
	0.24	0.26	0.256	84.5	0.22	0.240	89.2
	0.12	0.12	0.140	75.5	0.12	0.132	70.2
	0.06	0.06	0.071	68.5	0.05	0.058	58.5
	0.03	0.04	0.038	68.5	0.04	0.036	60.5
Spergon–DDT	0.48	0.47	0.502	83.0	0.44	0.486	86.2
	0.24	0.26	0.279	77.0	0.21	0.234	83.8
	0.12	0.12	0.135	74.3	0.12	0.134	80.5
	0.06	0.06	0.071	67.8	0.05	0.061	61.8
	0.03	0.03	0.037	61.5	0.04	0.038	54.8
Spergon–Wettable	0.48	0.48	0.445	79.3	0.44	0.412	84.2
	0.24	0.26	0.238	74.0	0.22	0.215	76.2
	0.12	0.12	0.115	66.3	0.12	0.117	66.2
	0.06	0.06	0.060	58.8	0.05	0.056	47.5
	0.03	0.03	0.028	48.5	0.03	0.029	51.2
None	... ...	0.00	0.000	15.8	0.00	0.000	37.5
None	. ...	0.00	0.000	16.0	0.00	0.000	35.8
None	..... .	0.00	0.000	18.0	0.00	0.000	34.2
Minimum Sign. Diff. at 5 per cent				6.4			7.9
Minimum Sign. Diff. at 1 per cent				8.4			10.5

[a] Dosages determined by analytical method corrected for percentage of active ingredients based on triplicate analyses.

as large as those between the results of weighing and analyses given in table 2. The deviations are primarily attributable to errors in the weighing of large samples of seed to 0.01 gm.

The practicability of the colorimetric method was tested to a more limited extent on seed of lima beans (var. Fordhook), spinach (var. Heavy Pack), and beets (var. Asgrow Canner). Seed were treated at the rate ordinarily used and the dosage determined by reweighing. The colorimet-

TABLE 3.—*Results of analysis of Spergon on beet, lima bean, and spinach seed*

Type of seed treated	Desired dosage of Spergon	Calculated dosage	
		By weighing	By analysis[a]
	Per cent	Per cent	Per cent
Lima beans	0.22	0.22	0.21
Spinach	0.50	0.51	0.50
Beet	1.00	0.95	0.92

[a] Average of three analyses, corrected for percentage of active ingredient.

ric analyses were conducted as described for corn and peas except that the size of samples was varied, as indicated in table 1, and a minor correction was necessary for pigments extracted from beets. This was made by extracting untreated seed in acetone and measuring the absorption of the solution in the presence of diethylamine. The data presented in table 3 show that the analytical method was applicable to these seed.

DISCUSSION

Although the method described in this paper is convenient and reliable, certain precautions must be observed in its use. For most purposes, satisfactory results can be obtained from duplicate samples. In the tests under discussion 10 to 20 seed (5 gm.) of either peas or corn were representative of the entire population. It is entirely possible, however, that larger samples may be required for poorly treated seed where the chemical is not so uniformly distributed. If larger samples are used a proportional increase in the amount of acetone will be necessary.

In adapting this method to commercial use where a minimum of calculations may be preferred, the following procedure can be substituted. A carefully weighed sample of the commercial product to be applied to the seed should be dissolved in acetone and diluted to a range of concentrations. These samples are used for standardizing the available equipment. The readings obtained from colorimeters that record data in optical densities can be plotted on rectangular graph paper against the known concentrations of chemical and the best straight line drawn through these points. Data from instruments recording percentage of light transmitted must be plotted on semi-logarithmic paper in order to obtain a straight line. Readings from unknown samples can be quickly transcribed to milligrams of chemical directly from these graphs.

SUMMARY

A simple colorimetric method of analyzing seed for tetrachloro-parabenzoquinone has proved exceptionally reliable on pea, corn, lima bean, spinach, and beet seed treated at various dosage levels over a range of one-fourth to four times the recommended concentration. The method consists of washing the chemical from the seed with acetone, adding diethylamine, and measuring the amount of light absorbed by the yellow reaction product that is formed. The optical density is directly proportional to the amount of tetrachloro-para-benzoquinone dissolved from the seed.

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PHYTOPATHOLOGICAL NOTES

A Valsa Associated with Cytospora Canker of Spruces.[1]—A canker disease of spruces was described by Gilgut and Boyd[2] in 1933. The causal fungus was identified tentatively by Dr. L. E. Wehmeyer,[3] as *Cytospora Kunzei* Sacc. No perfect stage of the fungus was observed on spruce.

Recently a species of *Valsa* was found associated with cankers on Colorado blue spruce (*Picea pungens* Engelm. var. *glauca* Beissm.), and Norway spruce (*Picea abies* [L.] Karst.). The writer believed the fungus to be *Valsa Kunzei* Fr., the perfect stage of *Cytospora Kunzei* Sacc., which has been reported as the cause of the spruce canker disease. A specimen was sent to Dr. L. E. Wehmeyer at the University of Michigan who stated that the fungus is the Leucostoma type of *Valsa,* which he has called *Valsa Kunzei* Fr.

The perithecial stromata of *Valsa Kunzei* which occur on cankered spruce branches are fairly widely scattered, rarely confluent, often interspersed with pycnidial stromata. They are seated in the cortex, are circular to fusoid, pustulate, erumpent, greyish-black, and measure 1.5–2.5 mm. in diameter. The ectostroma is truncate conical, and is surmounted by a greyish disc which is 0.5–.75 mm. in diameter and bears numerous black ostioles.

The perithecia, 7–20 in number, are seated closely together in the entostroma. They are globose, leathery, yellowish to black, with long converging necks. Exclusive of the necks the perithecia measure $431–631 \times 447–616 \mu$. The necks measure $92–169 \times 616–1032 \mu$, and are lined with delicate hyphae. Paraphyses are lacking.

The asci are numerous, broad-clavate, sessile or subsessile, hyaline, eight spored, and measure $5–7 \times 20–30 \mu$.

The ascospores are biseriate or bunched in the ascus, allantoid, hyaline, and measure $2.5 \times 5–7 \mu$.

Upon germination on potato-dextrose agar the spores increase to an average size of $12 \times 16.5 \mu$. They become nearly oval, oblong, or sometimes dumbbell-shaped. Two or more oil droplets are usually visible. Germ tubes are formed at both ends of most spores; less frequently one or three tubes are formed. The hyphae are hyaline and septated at unequal intervals. Irregular branching of the hyphae begins 18–24 hours after germ-tube formation.

Single-ascus and single-ascospore cultures on potato-dextrose agar and spruce-decoction agar were compared with pycnospore cultures of the *Cytospora* which causes the spruce canker disease. The ascospores and the

pycnospores both gave rise to a dull white or cream-colored, appressed mycelium, roughly circular or with fans, and with an irregular-crenate margin. Faint concentric zones of light and darker shades were formed. Considerable variation in coloring occurred on the same medium. Seven to ten days after inoculation numerous minute olivaceous bodies were formed on the surface of the medium. Twenty-five to 30 days after inoculation some of these bodies had enlarged to 1 mm. or more in diameter. Cross sections showed them to be rudimentary pycnidia, each consisting of a firm black center, tufted with grey hyphae, and containing hyaline, allantoid spores. None of the pycnidia matured enough to expel spore cirri.

The growth and appearance of the *Valsa* and the *Cytospora* were nearly identical on steam-sterilized twigs of Colorado blue spruce and Norway spruce. The mycelia grew from the cut ends and from the needle cushions in mats of white hyphae. Twenty-five to 30 days after inoculation superficial, globose, greyish pycnidia had been formed which bore hyaline, allantoid pycnospores, $2 \times 6–7 \mu$. Mycelial growth was slightly more luxuriant on Colorado blue spruce than on Norway spruce; more and slightly larger pycnidia developed on those twigs inoculated with the *Cytospora* than on those inoculated with the *Valsa*.

The pathogenicity of the *Valsa* was tested by inoculating five-year-old Norway spruce trees in the greenhouse. On April 24, 1947, ten lateral branches and one trunk were inoculated with mycelium from single-ascospore cultures. For each inoculation a razor cut was made in the bark and the mycelium placed therein. The inoculations were wrapped with moist cotton and waxed paper. The wrappings were left intact for one week. Six control inoculations with sterile agar were made in the same manner. On July 29, 1947, five of the inoculated branches were dead, each having been girdled by a resinous canker at the point of inoculation. Two other inoculated branches were cankered but alive. The remaining three were healthy with no signs of cankers. In the case of the trunk inoculation resinosus occurred, and a canker had been produced which extended halfway around the tree. No ill effects were apparent in the crown of this tree. No fruiting bodies had been formed on any of the inoculated trees. The fungus was re-isolated from each of the cankered branches. All of the control branches remained healthy and the razor wounds had healed.

Although a positive relationship was not established between the species of *Cytospora* which causes the canker disease of spruces and the species of *Valsa* which was found associated with cankers on spruce, the similarity in growth characteristics on nutrient media and sterilized twigs indicates that the *Valsa* may be the perfect stage of the *Cytospora*. Limited experiments have shown that cankers and death of Norway spruce branches may follow inoculation with mycelium from single-spore cultures of the *Valsa*. However, sufficient fertile perithecia were not found to determine the importance of the ascospores in the natural spread of the disease.—DAVID H. MARSDEN, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.

Low Temperature As a Factor in the Germination of Dwarf Bunt Chlamydospores.[1]—One of the characteristics of dwarf bunt (*Tilletia caries* (DC.) Tul.) of winter wheat is the difficulty encountered when trying to induce the chlamydospores to germinate in the laboratory.[2] Investigations[3] have shown that spores of this fungus may be induced to germinate by soaking them in water for several months prior to incubation on water agar.

Up to 30 per cent germination has been obtained by this method when the spores were incubated at 10° C., but no germination has been observed at higher temperatures. This fact, and the fact that the dwarf-bunt fungus attacks only wheat planted in the fall, suggested the possibility that the spores require a still lower temperature for germination. Investigations in line with this hypothesis were carried out and are reported here.

The materials used are from collections of dwarf bunt, from the Pacific Northwest,[4] which were stored at room temperature for periods ranging from 3 to 13 years. All germination tests reported were made in 1947, taking spores directly from storage without any pre-soaking.

Germination tests were made on 4 per cent water agar in Petri dishes at temperatures of 0°, 5°, and 10° C. Observations were made weekly to determine the progress of germination. Percentages of germination after 49 days were determined by estimate while those on later dates were determined by actual count. No attempt was made to determine the percentage of germination after 75 per cent of the spores had germinated because by then the colonies were too closely intermingled. The experiment was discontinued after 104 days, as further increases in germination could not be detected after that time.

The results of this test are presented in table 1. These data indicate that the optimum temperature for germination was 5° C. Germination began at the end of the fifth week. Percentage germination determinations, however, were made only at the three different times, as shown in table 1.

At the end of 49 days up to 50 per cent germination had occurred in all collections, and this increased to 75 per cent at the end of 70 days. The period of germination, therefore, extended over about five weeks, whereas in common bunt it extends over a period of but a few days.

In these tests no germination was observed at 10° C. In some other tests a high percentage germination was obtained at 0° C., after a period of three months. The results of all tests indicate that the optimum temperature for spore germination in dwarf bunt under the conditions described is approximately 5° C. When incubated a few degrees above or below this

[2] Holton, C. S. Preliminary investigations on dwarf bunt of wheat. Phytopath. 31: 74–82. 1941.

[3] Holton, C. S. Chlamydospore germination in the fungus causing dwarf bunt of wheat. Phytopath. 33: 732–735. 1943.

[4] All materials used in these tests were obtained from Dr. C. S. Holton, to whom the writer is indebted for aid and encouragement during these and related studies.

temperature, there seems to be a marked reduction in both the percentage and the speed of germination. In preliminary tests of several 1947 collections of dwarf bunt spores, good germination was observed under the same

TABLE 1.—*The percentage germination of dwarf-bunt chlamydospores after three different periods of incubation on 4 per cent water agar at three different temperatures, Centigrade.*

Place and year collected	After 49 days			After 70 days			After 104 days		
	0°	5°	10°	0°	5°	10°	0°	5°	10°
Plummer, Idaho—1938	0	25–50	0	0	75	0	10	75	0
Cache valley, Utah—1934	0	25–50	0	0	75	0	2	75	0
Cache valley, Utah—1944	0	10–25	0	0	50	0	3	50	0
Nez Perce, Idaho—1937	0	25–50	0	0	75	0	3	75	0
Troy, Idaho—1940	0	25–50	0	0	40	0	2	50	0
Troy, Idaho—1944	1–5	25–50	0	10	75	0	20	75	0
High Prairie, Wash.—1940	0	25–50	0	0	75	0	2	75	0
Kalispell, Montana—1942	0	25–50	0	3	60	0	40	75	0

conditions, indicating that age is not a factor in the germination of these spores.—CONLEY V. LOWTHER, Department of Plant Pathology, Washington State College, Pullman, Washington.

Ringspot of Papaya (Carica papaya) in the Hawaiian Islands.—Papaya ringspot, a previously undescribed disease, was discovered in the Territory of Hawaii at Kailua, Oahu, in March, 1945. Its occurrence and general symptoms were recorded by Lindner et al.[1] Subsequently, experiments reported by Jensen[2] demonstrated that papaya ringspot is caused by a virus which is transmissible by the aphid *Myzus persicae* (Sulzer). In these experiments symptoms developed in papayas in from 11 to 21 days after inoculation.

The most distinctive and reliable symptom of the disease appears on the fruits as they approach maturity. The surfaces of such fruits, while still mostly green, develop yellow rings with green centers. The rings vary from ¼ to ¾ of an inch in diameter.

Foliage symptoms of the disease include elevation, or puckering, of the leaf tissue between the veins and veinlets of the youngest leaves and irregular mosaic patterns in expanded leaves. The mosaic symptoms are most pronounced in field trees during the winter season, becoming masked in varying degrees during the summer.

Because papaya ringspot produces typical mosaic symptoms in the foliage, the disease could appropriately be called papaya mosaic. However, the name papaya mosaic has already been used for another papaya disease in Hawaii which Parris[3] reported to be due to a virus. Moreover, Ho and Li[4]

[1] Lindner, R. C., D. D. Jensen, and W. Ikeda. Ringspot: New papaya plunderer. Hawaii Farm and Home 8(10): 10, 12, 14. 1945.

[2] Jensen, D. D. Virus diseases of plants and their insect vectors with special reference to Hawaii. Proc. Hawaiian Entomol. Soc. 12: 535–610. 1946.

[3] Parris, G. K. A new disease of papaya in Hawaii. Proc. Amer. Soc. Hort. Sci. 36: 263–265. 1938.

have applied the name papaya mosaic to a virus disease of papayas in China, and Baker[5] has used the term papaw mosaic for a virus disease of papayas in Trinidad.

The origin of papaya ringspot is not known. Its restricted distribution, despite rapid spread within plantings that have become infected, seems to indicate that the disease, in its present form, became established in the Territory within recent years.

Papaya ringspot virus rarely, or perhaps never, kills infected papaya plants. However, the disease appears to retard the growth of plants and weaken them. In areas of high disease incidence commercial papaya production has been appreciably affected.

A survey of the island of Oahu was made in the spring of 1945, in cooperation with the Board of Agriculture and Forestry to determine the distribution of papaya-ringspot disease. The disease was found to be limited to a relatively small portion of the island. The Kailua-Maunawili district, near the eastern end of the island, was found to have a fairly high percentage of diseased papaya plants. On one farm, nearly 100 per cent of the papayas were affected. The incidence of disease was observed to diminish progressively to a 20 per cent level within a half-mile of this point. The distribution from this heavily infested area was mostly to leeward (*i.e.*, to the southwest). Five scattered infestations were found in the Kokokahi district, about 3 miles to the north of the heavily infested Kailua district. In two of these, the papaya ringspot could be traced to use of papaya seedlings originally grown in the Kailua area and subsequently transported to the Kokokahi district for planting. Diseased plants were also found scattered throughout the city of Honolulu, which is directly to the leeward side of the Kailua area, but across the Koolau range of mountains that vary in this region from 1200 to 3100 feet in elevation.

Spread of disease within individual plantings proved rapid during the winter and spring months, but slow during the summer months. This is presumed to be a result of seasonal abundance of aphid vectors.

During the latter part of 1945 the disease progressed rapidly in the direction of prevailing winds within the Kailua-Maunawili district but there has been little lateral spread. In the spring of 1946 the area of essentially complete infection had increased from the one farm to include a number of others within a half-mile radius with proportionate increases in the area immediately to the southwest.

The possible existence of papaya ringspot on islands of the Territory other than Oahu was investigated during June and July of 1946, when a survey of Kauai, Maui, Molokai, and Hawaii was made. Principal attention was given to climatologically diverse areas where papayas had been culti-

    [4] Ho, W. T. H., and L. Y. Li. A virus disease of papaya (*Carica papaya* L.). Preliminary notes on the virus diseases of some economic plants in Kwangtung Province. Lingnan Sci. Jour. **15**: 67–78. 1936.
    [5] Baker, R. E. D. Papaw mosaic disease. Trop. Agr. (Trinidad) **16**: 159–163. 1939.

vated for long periods of time in commercial and in dooryard plantings. Representative plantings within the immediate vicinity of airports and seaports were given special consideration since the introduction of a new disease would be especially likely in such areas. In no part of any of the four islands included in this survey was the disease found.

The possibility of control of papaya ringspot, as it occurs in the Territory of Hawaii, would seem to be dependent on the applicability of one or more of four commonly used procedures. These are (1) control of insect vectors, (2) substitution of immune or highly tolerant varieties of papaya for those now grown, (3) interpolation of crop-free periods, and (4) destruction of diseased trees.

Control of insect vectors would mean control of aphids, especially of the aphid *Myzus persicae,* in the case of papaya ringspot. Aphicides are effective but expensive and not commonly used by papaya growers in Hawaii. Moreover, *Myzus persicae* breeds on many species of plants and would tend to reinfest papaya soon after treatment of this plant.

Substitution of immune or highly tolerant varieties of papaya for those now grown offers no immediate prospect of solving the problem. At present no variety immune from the disease is known. About 15 varieties, varietal strains, and hybrids, representing material from both local and foreign sources, have been grown in areas where the disease prevails. In every instance, this material has contracted the disease through natural spread.

Crop-free periods of the usual seasonal type would be inapplicable to the problem of control in the case of a papaya disease, because the host is grown as a short-lived perennial in overlapping succession. It would be necessary to induce growers to omit succession planting for a period long enough to provide a wholly crop-free interval.

Destruction of diseased trees sometimes seems uneconomical to owners, who may prefer to continue picking fruit as it matures rather than attempt control by eradicating the reservoir of disease within the planting. Control by removal of diseased trees, however, would be favored by many circumstances. Not enough trees are involved by disease, as yet, to affect appreciably the current supply of fruits for market. The papaya plant is readily cut down and does not regenerate from roots left in the ground if all stem tissues are removed by the initial cut. It rarely grows as a weed in the Territory but is essentially confined to cultivated plantings and to dooryard plots, where it is readily found. Thus far no other species of plant has been found capable of acting as host of the causative virus.

In view of the considerations discussed above, removal of diseased trees would seem to provide the best basis for a program for control of papaya-ringspot disease, other procedures being considered as of possible supplementary value whenever applicable.—F. O. HOLMES, The Rockefeller Institute for Medical Research, Princeton, N. J.; J. W. HENDRIX, W. IKEDA, D. D. JENSEN, R. C. LINDNER, and W. B. STOREY, University of Hawaii Agricultural Experiment Station, Honolulu, T. H.

REPORT AND ABSTRACTS OF THE SECOND ANNUAL MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The second annual meeting of the Northeastern Division of the American Phytopathological Society was held in Poughkeepsie, New York, November 25–26, 1947, immediately following the New England–New York Spray Specialists' Conference. Approximately 70 persons attended the meeting. Discussion meetings were held on the following topics: 1947 Cooperative Fungicide Tests for Apple Scab Control, Classifying Scientific Data for Punch Cards, Turf Diseases and Their Control, Fungicide Control of Potato Diseases, and Diagnosis of Plant Diseases. Ten formal papers were presented at one afternoon session. At a short business meeting the new constitution was adopted. The following officers were elected for 1948:

> President: O. C. Boyd
> Vice President: D. H. Palmiter
> Secretary-Treasurer: L. M. Black
> Councilor: S. E. A. McCallan

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Relation of insecticides to the development of diseases of ornamentals. A. W. DIMOCK. The development of leafspot (*Septoria obesa*) in the upper foliage of chrysanthemums was observed under conditions which precluded spore dissemination by splashed water, the only efficient dispersal mechanism then recognized. In all cases the plants had been sprayed with rotenone insecticides. Viability tests of spores suspended briefly in spraying-strength emulsions of such insecticides, and actual inoculations with the suspensions, proved that viable inoculum could be spread from infected basal leaves to the upper foliage by such sprays. Of 11 materials tested, some showed very high, others very low fungicidal value. Although spray droplets of such suspensions may evaporate too rapidly to permit immediate infection, this may occur subsequently as a consequence of nightly deposition of dew. An unusual epidemic of blackspot (*Diplocarpon rosae*) developing abundantly on the upper, but as only a trace on the lower, foliage of greenhouse roses was observed. Spores had been disseminated to the upper foliage by syringing with water for red spider control, though the water had evaporated too rapidly for infection to occur. A subsequent application of tartar emetic and sugar, fogged on the upper leaves for thrips control, provided a hygroscopic deposit retaining sufficient condensed moisture long enough to permit infection.

Common blight of bean as a screen for testing chemotherapeutic activity. A. E. DIMOND and E. M. STODDARD. Common blight of bean (*Xanthomonas phaseoli*) was found to be a good medium in screening chemicals for therapeutic activity. Systemic infections produced sharp differences between good treatments and checks. Red Kidney beans were grown in sand and fertilized to give maximum symptoms of disease. Systemic infection was produced by inoculating the stem with bacterial stabs. A solution of the chemical to be tested, just non-phytotoxic in concentration, was applied to the potted plants on the nine days following the inoculation period. Two weeks after inoculation plants were rated for incidence of disease as measured by percentage of infection, and severity of symptoms as measured by a disease index. Typical data for percentage of infection and disease index follow: $KMnO_4$—100 and 3.48; $CaCl_2$—86 and 1.40; $ZnSO_4$—61 and 1.17; Hydroquinone—89'and 2.05; Auramine—58 and 0.57; Salicylic acid—14 and 0.17; Check—100 and 3.38. Tests so far conducted indicated greater success when chemicals were applied after rather than before inoculation.

Evaluation of organic fungicides for the control of Venturia inaequalis (Cke.) Wint. J. M. HAMILTON. Most organic fungicides have given mediocre or erratic performance in field tests. The testing of organics on potted greenhouse-grown trees as to retention and eradicative action at the time infection occurs has aided in the interpretation of their performance. Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate), 806 (phenyl mercury compound), and PMAS (phenyl mercury salt) were comparable to liquid lime-sulfur in the length of time that they controlled scab after infection began. Phygon (2,3-dichloro-1,4-naphthoquinone), copper 8-quinolinolate, and manganese ethylene bis dithiocarbamate were moderately effective in the order of their inherent toxicity. The presence of moisture to release the toxicants after their applica-

tion markedly increased their effectiveness. This was not true with the Puratized group. Fermate (ferric dimethyl dithiocarbamate), Dithane Z78 (zinc ethylene bis dithiocarbamate), and the glyoxalidine materials were not eradicative and functioned mainly as preventives. Good-rite P.E.P.S. (polyethylene polysulphide) was adhesive but did not redistribute. The work supported the trend in field data in that the organics with the greatest retention were superior in seasons where sprays could be timed. On the other hand, eradicative type materials were most effective in periods of more or less continuous precipitation. A mixture of an eradicative material with a protective fungicide has merit.

Factors influencing the control of carrot yellows with DDT. G. E. R. HERVEY and W. T. SCHROEDER. The vector of carrot yellows in western New York, *Macrosteles divisus* (Uhl.), occurs and breeds on carrots from the time the plant emerges until harvest. In 1946 and 1947 there was a definite peak in vector population, beginning when the carrots were three to five inches tall and extending for 25 to 30 days. This peak resulted almost exclusively from migrating adults. The possibility that factors other than vector population may influence the amount of yellows was indicated by the fact that in comparable nontreated plots, the leafhopper population in 1946 was three times as great as in 1947, yet the disease was slightly higher the latter year. DDT applied during the peak immediately reduced the insect population to practically nil, but it built up rapidly by migration. Dusts were less effective than sprays, and the latter were more effective when used with an adhesive. Plots treated with DDT showed no nymphal development for as much as 8 weeks after the last application. DDT applications timed to coincide with the beginning of the peak in vector population gave as good or better control with fewer applications than when this factor was ignored.

Elimination of spotted wilt from a stock of dahlia. FRANCIS O. HOLMES. An unusually severe outbreak of spotted wilt in dahlia occurred near Vineland, New Jersey, during 1946. The Rhythm dahlia was most severely affected of all varieties observed, and every available plant was diseased. In this dahlia, as in all others, lower leaves were more damaged than upper ones. Shoots grown from diseased roots generally appeared healthy at first, though if left attached to the old roots they showed lesions of the disease eventually. Tip cuttings taken from emerging shoots were readily rooted. Only a few of these showed disease when established as potted plants. A majority of the rooted tip-cuttings remained healthy in appearance and were grown to blossoming in the greenhouse under spring season conditions without showing any evidence of spotted wilt. Thirty of the apparently healthy cutting-plants also grew vigorously throughout the summer in a field plot. In no case did the disease reappear. It was concluded that this stock of Rhythm dahlia had been freed of virus by radical removal of diseased tissues, *i.e.*, old roots, stems, and lower leaves.

A comparison of aircraft dusting and ground spraying for the control of tomato diseases. G. L. MACK and W. T. SCHROEDER. Six alternate applications of Zerlate (zinc dimethyl dithiocarbamate) and Tribasic copper sulfate (54 per cent) were applied to tomatoes as dusts with a Bell helicopter and with a Stearman fixed wing aircraft, and as sprays with a trailer-drawn brush-boom spray rig. Application rates were adjusted to deliver 4 lb. Zerlate or 4 lb. metallic copper per acre. Ten-row plots, each approximately 1.7 acres in area and separated from each other by sprayed five-row buffer plots were randomized in each of three blocks. Data were obtained only from the five center rows of each plot. Following the fourth application, deposition and distribution of copper on the upper and lower surfaces of twenty leaves, selected at random from each plot at three different positions on the plant were determined by a print method and analyzed statistically. Coefficients of variability indicated a much more even distribution of the fungicide with the ground sprayer than with either of the aircraft applicators. Copper deposit ratings were related significantly to total yield and late blight control. · Significant differences between the three applicators in yield and late blight control ranked the ground sprayer first, followed by the helicopter and aeroplane. Anthracnose control was significantly better with the ground sprayer than with either of the aircraft applicators.

Apple scab control with elemental sulfurs in the Hudson Valley in 1947. W. D. MILLS and J. D. VANGELUWE. Six formulations of air-ground sulfurs were compared with Magnetic ''70'' sulfur paste, Troy Flotation sulfur paste, and micronized sulfur, using 2½ lb. actual sulfur per 100 gal. on McIntosh and Delicious varieties. One formula of air-ground sulfur was compared with the paste sulfurs and micronized sulfur at 5 lb. actual sulfur. Sprays were timed by bud development and rain forecasts. Two of the applications were made at the beginning of infection period rains. Treatments at 2½ lb. per 100 gal. were remarkably good in view of the heavy rains between sprays. The two paste types of sulfur were equally effective. Both pastes and one formulation of air-

ground sulfur were significantly better (99: 1) in fruit scab control than micronized sulfur when 5 lb. actual sulfur were used on both McIntosh and Delicious. The control of fruit scab on Delicious by 1 and 1½ lb. Fermate (ferric dimethyl dithiocarbamate) equalled that obtained with the 5-lb. dosage of the better elemental sulfurs, and was significantly better (99: 1) 'han micronized sulfur.

Some effects of fungicides on yield, quality, and maturity of apples in 1947. D. H. PALMITER. Phygon (2,3-dichloro-1,4-naphthoquinone)—¾ lb. Phygon—⅛ lb. plus Goodrite P.E.P.S. (polyethylene polysulphide)—1 lb., Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate)—1 pt., Fermate (ferric dimethyl dithiocarbamate) —1½ lb., copper 8-quinolinolate—1 lb., Micronized Sulfur—5 lb., and 341C (2-heptadecyl glyoxalidine)—1 qt., each in 100 gal. water and applied to McIntosh in seven sprays, held fruit infection from apple scab to less than five per cent. Manganese ethylene bis dithiocarbamate at 1½ lb. and P.E.P.S. at 2 lb. per 100 gal. did not afford as good control as the other materials. DN–111 (dicyclohexyl amine salt of dinitro-orthocyclohexyl-phenol) severely injured the foliage and caused fruit drop when applied one month after an application of Phygon. Trees sprayed with 341C showed foliage injury in the form of purple veins and a reduction in leaf size and color. Fruits from these trees were smaller than average. Trees in the Puratized plots matured their fruit a week later than the rest of the orchard and had the best color and quality. Russet injury on similarly sprayed Greening plots was very severe with 341C, moderate with Sulfur, Fermate, and P.E.P.S., slight with copper 8-quinolinolate and Puratized, and almost absent with Phygon and Manganese carbamate.

Air blast application of oil-soluble fungicides to row crops. J. B. ROWELL and F. L. HOWARD. Modern air blast methods of pesticide application require a reduced quantity of carrier. Likewise, fungicides compatible with the new oil-soluble insecticides are needed for simultaneous application. Quantitative data on the specifications needed for implementing this principle of disease control were obtained by experimenting with a modified Potts-Spencer "Mist Blower" mounted on a Bolens HiBoy row-crop two-wheel tractor. Particle size and type of oil are of major importance in safeness on potato and tomato foliage. A wide distribution pattern induced by turbulence is essential for uniformity of coverage and avoidance of injury. Oil-soluble fungicides were applied with slight or no injury to field plots of potato, tomato, cucumber, and snap bean. Bean anthracnose control was demonstrated with Mycotox-4 (substituted phenyl ester) and Onyx DL-1 (didodecyl dimethyl ammonium bromide) carried in 3 gallons of Shell Horticultural base oil No. 7 per acre. Cucumber downy mildew infection was decreased 25 per cent by fungicide-oil mist treatment.

Susceptibility to common blight of bean as influenced by level of nutrition. E. M. STODDARD. The effect of the nutrition level on the susceptibility of Red Kidney bean to common blight (*Xanthomonas phaseoli*) was studied on plants grown in sand at nutrient level increments of 1, 10, and 100. The nutrient solution used contained potassium nitrate, monopotassium phosphate, calcium nitrate and magnesium sulfate. Inoculation was accomplished by stabbing the causal organism into the stem. The data were recorded 13 days after inoculation. The percentages of plants showing wilting of the foliage at the above ascending order of nutrient levels were 91, 88, and 21. The inoculated plants at the highest nutrient level showed more stunting than either those at the lower levels or the checks. The causal organism was isolated from the plants not showing wilting at the highest level, indicating that these plants tolerated the organism without exhibiting the usual wilting symptoms.

REPORT OF THE 39TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 39th annual meeting at the Stevens Hotel, Chicago, Illinois, December 28–30, 1947, in connection with the American Association for the Advancement of Science. Three hundred and thirteen members from forty-two states and three foreign countries registered. One hundred and twenty-seven papers, accepted by the editorial committee, were presented at the meeting. The sections and number of papers presented in each follow: diseases of corn and small grains, 13; diseases of fruits and ornamentals, 13; physiology of fungi, 12; diseases of field crops, 10; virus diseases, 11; breeding and disease resistance, 10; joint session with the Potato Association of America, 5; antibiotics and bacterial diseases, 13; virus diseases of vegetables, 6; fungicides, 10; vegetable diseases, 11; and miscellaneous, 13.

A joint symposium was held with the Committee on Genetics of Microorganisms of the National Research Council, the American Society of Naturalists, the Mycological Society of America, and the Microbiological Section of the Botanical Society of America. A joint session was also held with the Potato Association of America.

Conferences included "Fungicide Colloquium," "Extension Workers," "Plant Disease Survey," "National Security," and "Upper Mississippi Valley Plant Pathologists."

There were demonstration sections on Market Diseases of Fruits and Vegetables, and Publicity.

The premiere showing of the color, sound, moving picture "White Pine Blister Rust Control" was presented by the United States Department of Agriculture.

The Phytopathologists' dinner, held at the Chicago Bar Association, on Monday evening, December 29, was attended by over three hundred.

Council for 1948:

R. S. KIRBY, President (1 yr.), Pennsylvania State College, State College, Pennsylvania.

W. D. VALLEAU, Vice-President (1 yr.), Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

CURTIS, MAY, Secretary (3-yr. term expires 1950), Plant Industry Station, Beltsville, Maryland.

M. C. RICHARDS, Treasurer, and Business Manager of PHYTOPATHOLOGY (3 yr. term expires 1949), University of New Hampshire, Durham, New Hampshire.

HELEN HART, Editor-in-Chief, PHYTOPATHOLOGY (3-yr. term expires 1948), University Farm, St. Paul 1, Minnesota.

E. E. CLAYTON, Plant Industry Station, Beltsville, Maryland.

MAX W. GARDNER, University of California, Berkeley, California.

J. H. JENSEN, North Carolina State College, Raleigh, North Carolina.

S. G. LEHMAN, North Carolina State College, Raleigh, North Carolina.

S. E. A. McCALLAN, Boyce Thompson Institute, Yonkers 3, New York.

S. J. P. CHILTON, Louisiana State University, Baton Rouge, Louisiana.

A. J. RIKER, University of Wisconsin, Madison, Wisconsin.

Representatives:

A.A.A.S. Council. R. W. GOSS, H. A. RODENHISER.
Division of Biology and Agriculture, National Research Council. J. C. WALKER.
Board of Editors, American Journal of Botany. CHARLES CHUPP.
American Institute of Biological Sciences. J. C. WALKER.

Standing Committees:

Donations and Legacies. H. S. FAWCETT, P. P. PIRONE, R. E. VAUGHN, A. A. DUNLAP, W. B. TISDALE, N. J. GIDDINGS, Chm.

Extension. O. C. BOYD, O. D. BURKE, L. O. WEAVER, R. J. HASKELL, J. O. ANDES, S. B. FENNE, JOHN R. VAUGHN, W. J. HENDERSON, E. G. SHARVELLE, J. T. MIDDLETON, T. H. KING, Chm.

Investments. MARVIN E. FOWLER, E. L. LECLERG, NEIL E. STEVENS, R. M. CALDWELL, M. C. RICHARDS, Chm.

Phytopathological Classics. L. J. TYLER, Business Manager; L. C. KNORR, Editor.
Placement. H. A. RODENHISER, M. W. GARDNER, R. J. HASKELL, L. M. MASSEY, S. J. P. CHILTON, R. S. KIRBY, Chm.
Public Relations. O. D. BURKE, L. S. HITCHNER, J. D. MOORE, A. G. NEWHALL, HARRY R. O'BRIEN, Γ. J. GREANEY, H. T. COOK, J. E. LIVINGSTON, E. C. COX, R. R. KINCAID, K. S. ARR CHESTER, Chm.
International Cooperation. G. H. COONS, K. G. KEVORKIAN, I. E. MELHUS, M. H. LANGFORD, J. G. HARRAR, J. A. STEVENSON, OTTO REINKING, H. P. BARSS, E. C. STAKMAN, Chm.
Society Organization. W. J. ZAUMEYER, A. L. HARRISON, L. W. KOCH, J C. WALKER, O. C. BOYD, GEORGE ARMSTRONG, Chm.
Plant Disease Prevention. PAUL R. MILLER, S. B. FRACKER, E. C. STAKMAN, H. H. THORNBERRY, D. G. FLETCHER, J. J. CHRISTENSEN, Chm.
Subcommittee on Regulatory Work and Foreign Plant Diseases. C. R. ORTON, R. P. WHITE, E. C. STAKMAN, Chm.
Subcommittee on Seed and Plant Material Certification. REINER BONDE, L. C. COCHRAN, CHARLES CHUPP, C. H. ARNDT, J. J. CHRISTENSEN, Chm.
Subcommittee on Utilization of Plant Pathologists and Field Facilities in National Emergencies. M. W. GARDNER, LESLIE WALDEE, H. T. COOK, H. H. THORNBERRY, Chm.

Special Committees:

Fungicides. C. H. ARNDT, J. D. WILSON, J. W. HEUBERGER, F. L. HOWARD, DWIGHT POWELL, C. E. YARWOOD, J. G. HORSFALL, Chm.
Subcommittee on Seed Treatments. E. D. HANSING, H. T. COOK, GEORGE SEMENIUK, J. E. MACHACEK, C. H. ARNDT, Chm.
Subcommittee on Dusts and Sprays. J. M. HAMILTON, A. W. DIMOCK, A. G. NEWHALL, J. W. HEUBERGER, W. F. BUCHHOLTZ, H. F. WINTER, M. B. LINN, J. D. WILSON, Chm.
Subcommittee on Special Problems. L. S. HITCHNER, E. A. WALKER, J. G. LEACH, F. L. HOWARD, Chm.
Subcommittee on Annual Summary of Results on Newer Fungicides. PAUL R. MILLER, E. E. CLAYTON, M. C. GOLDSWORTHY, R. W. LEUKEL, R. J. HASKELL, W. D. McCLELLAN, J. W. HEUBERGER, Chm.
Subcommittee on Fungicide Nomenclature. J. D. MOORE, W. H. TISDALE, J. C. DUNEGAN, S. L. HOPPERSTEAD, E. G. SHARVELLE, DWIGHT POWELL, Chm.
Fungus Nomenclature. W. W. RAY, W. C. SNYDER, D. E. ELLIS, C. M. CHRISTENSEN, J. A. STEVENSON, Chm.
Nomenclature and Classification of Plant Viruses. FREEMAN WEISS, C. W. BENNETT, H. H. McKINNEY, JAMES JOHNSON, R. H. LARSON, H. R. McLARTY, FRANK McWHORTER, L. M. BLACK, Chm.
Subcommittee on Virus Type Culture Collection. JAMES JOHNSON, THEODORE J. GRANT, H. H. McKINNEY, Chm.
Membership. W. J. JEFFERS, T. W. BRETZ, B. H. DAVIS, C. M. CLAYTON, THOR KOMMEDAHL, GLENN S. POUND, C. M. TOMPKINS, Chm.
Sustaining Associates. J. D. WILSON, E. G. REX, P. D. PETERSON, E. G. SHARVELLE, FRED LEWIS, P. P. PIRONE, Chm.
Nomenclature of Plant Pathogenic Bacteria. J. G. LEACH, W. H. BURKHOLDER, CHARLOTTE ELLIOTT, E. M. HILDEBRAND, P. A. ARK, O. N. ALLEN, Chm.
Teaching of Plant Pathology. J. G. LEACH, G. C. KENT, C. M. TUCKER, M. F. KERNKAMP, G. F. WEBER, Chm.

Temporary Committees for 1948:

Membership List Committee. HELEN HART, M. C. RICHARDS, L. J. ALEXANDER, Chm.
Publication of Special Material. H. P. BARSS, J. G. LEACH, W. H. TISDALE, DONALD CATION, Chm.

Report of the Secretary. On November 5, 1947, the total membership was 1322, consisting of 145 life members, 1096 regular members, and 81 applicants to be inducted at the Chicago meeting of the Society. During the year, 45 members were suspended for nonpayment of dues, 3 died, and 12 resigned. Twenty-seven additional applications were received after November 5. The membership as of December 31, 1947 is about 1350.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1947.

Receipts:

Balance from 1946			$ 2,781.58
Annual dues:			
1946	$ 20.00		
1947	5,683.49		
1948	2,112.16		
1949	8.00		
1950	.50	$7,824.15	
30-Year Index		6.00	
Sales		68.00	
Membership List		.50	
1947 Subscription		6.50	
Surplus from 12/46 banquet		17.00	
Sustaining Associates		1,900.00	
Total receipts			9,822.15
			$12,603.73

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY			
1946	$ 16.00		
1947	4,537.64		
1948	1,759.23		
1949	6.62		
1950	.42	$6,319.91	
Transferred to PHYTOPATHOLOGY for:			
30-Year Index	6.00		
Sales	68.00		
Membership list	.50		
1947 subscription	6.50	81.00	
Office of Secretary			
Secretarial work	46.20		
Stamps	52.86		
Printing	245.14		
Other	13.21	357.41	
Office of Treasurer			
Secretarial work	540.80		
Supplies	73.17		
Express	94.11		
Bank charges	26.30		
Auditing	25.00		
Printing	4.24		
Other expenses	18.73	782.35	
Biological Abstracts	100.00		
Placement Committee	16.60		
Sustaining Associates Committee	10.00		
Total expenditures			7,667.27
Balance on hand September 30, 1947			4,936.46
			$12,603.73

Report of the Business Manager. During the past year the costs for printing PHYTOPATHOLOGY and reprints from PHYTOPATHOLOGY increased another 10 per cent, making a total over pre-war prices of 30 per cent for PHYTOPATHOLOGY and 20 per cent for reprints. Certain functions formerly done by the printer, such as typing of mailing slips and the billing of authors for reprints, were transferred to the Business Office.

Total non-member subscriptions on November 10, 1947, were 840, and adding to these the 1320 member subscriptions gives a total distribution for PHYTOPATHOLOGY of 2,160.

The sales of back volumes during 1947 continued high, amounting to $2,032.27.

Statement of accounts for the year ending September 30, 1947.

Receipts:

Balance from 1946			$ 9,980.81
Subscriptions:			
1946	$ 25.10		
1947	4,595.40		
1948	511.60		
1949	39.50	$5,171.60	
Member subscriptions:			
1946	16.00		
1947	4,537.64		
1948	1,759.23		
1949	6.62		
1950	.42	6,319.91	
Sales of back numbers of PHYTOPATHOLOGY		2,032.27	
Sales of Membership List		4.50	
Advertising			
1946	379.18		
1947	885.34	1,264.52	
30-Year Index		112.55	
Interest on current funds			
First mortgage	20.25		
Building and Loan	50.00		
U. S. Bond, Series G.	25.00	95.25	
Interest on current funds		127.51	
Grant from Rockefeller Institute		600.00	
Allowance on reprints		825.64	
From authors for excess illustrations		318.05	
Unused credit		38.90	
Membership dues		26.00	
Sustaining Associates		200.00	
Total receipts			17,136.70

Expenditures: $27,117.51

Printing, distributing, and storing PHYTOPATHOLOGY:

Vol. 36, no. 9	$740.82		
10	887.00		
11	768.79		
12	958.71		
Vol. 37, no. 1	703.68		
2	830.72		
3	631.43		
4	752.17		
5	987.52		
6	701.19		
7	861.72		
8	775.60		
Engravings, Sept. '46 to Aug. '47	944.76	$10,544.11	
Postage, PHYTOPATHOLOGY	929.53	$11,473.64	
Secretarial work and office expense, Editor-in-Chief		1,253.34	
do Advertising Mgr.		47.65	
Commission for Advertising Manager, 1946		92.50	
Office of the Business Manager			
Secretarial work		546.70	
Office supplies		38.14	
Stamps		180.47	
Miscellaneous		69.66	
Refund, subscriptions and sales		307.41	
Purchase, back volumes		215.50	
Express		33.97	
Printing		119.31	
Binding volumes		15.39	
Transfer, APS dues		45.50	
Transfer, APS Sustaining Assoc. contributions		200.00	
Miscellaneous		21.73	
Total expenditures			$14,660.91
Balance on hand:			
Checking account		7,259.93	
Northwestern Federal Savings & Loan (Wash., D. C.)		5,196.67	12,456.60
			$27,117.51

Sinking Fund. By the completion of payment on a life membership by an Italian member, the principle amount of the sinking fund was increased this year by $30.34, it now being $9,706.34.

First mortgage note, at 4½ per cent interest, deposited with McLachlen Banking Corporation for collection	$ 500.00
U. S. Savings Bond, Series G, No. M19056020, 2½ per cent	1,000.00
Invested with the following:	
Jefferson Federal Savings (formerly Columbia Permanent Bldg. Assoc.) (accrued dividends $85.96)	585.96
District Bldg. Loan Association (accrued dividends $253.67)	1,753.67
National Permanent Bldg. Assoc. (accrued dividends $390.03)	2,390.03
Northwestern Fed. Savings & Loan Assoc. (certificates)	2,000.00
Perpetual Building Association (accrued dividends $171.92)	1,171.92
Prudential Building Association (accrued dividends $38.69)	245.03
Arlington and Fairfax Bldg. & Loan (accrued dividends $77.26)	1,077.26
	$10,723.87
Less interest due PHYTOPATHOLOGY	1,017.53
	$ 9,706.34

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3,321.82, This amount is invested with the Brookland Building and Loan Association at 2¼ per cent. The account for 1947 is as follows:

Balance on hand, October 1, 1946	$ 3,703.80
Dividends, December 31, 1946 and June 30, 1947	93.16
Voluntary contributions	11.00
	$ 3,807.96
Less interest due PHYTOPATHOLOGY	486.14
	$ 3,321.82

Additional Endowment:

War Savings Bond, Series F	
Total, September 30, 1947	$1,125.00
War Savings Stamps	
Total, September 30, 1947	7.00
	$1,132.00

The 30-Year Index. Summary of receipts (funds deposited in PHYTOPATHOLOGY checking account) October 1, 1946 to September 30, 1947:

Balance (receipts less costs) September 30, 1946	$373.52
Receipts October 1, 1946 to September 30, 1947	112.55
Balance on hand, September 30, 1947	$486.07
Membership List Account, 1946–1947	
Balance on hand, October 1, 1946	$ 52.46
Membership lists sold	4.50
Balance on hand, September 30, 1947	$ 56.96

Report of the Auditing Committee, as of September 30, 1947. We have examined the books of the Treasurer of the American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period from January 22, 1947 to September 30, 1947. We found all receipts and expenditures and all funds of the Society and of PHYTOPATHOLOGY lucidly and accurately recorded.

Signed: STUART DUNN, *Chairman*

M. D. FARRAR

Report of the Advertising Manager. PHYTOPATHOLOGY carried a total of 94 pages of paid advertisements in 1947. The gross income from advertising was $1625.60. The net income to the Society was $1382.67. The sixteen companies which advertised in 1947 have been written concerning renewal of contracts for 1948. Six companies have contracted for 27½ pages for a total of $1260. Two other companies are likely to contract for 24 for a total of $928.80. Three companies have canceled

their contracts, and five have not replied. The slowness in new contracts is due to increased rates and to the fact that some contracts do not expire until early 1948. It now appears that there will be fewer advertisers in 1948, but the income will possibly be more than in 1947.

Report of the Editor-in-Chief. Volume 37 of PHYTOPATHOLOGY contained only 931 pages, but 335 authors used these pages in the publication of 89 long articles, 37 phytopathological notes, 209 abstracts, 2 biographies, 2 committee reports, and 8 book reviews. The report of the annual meeting, the revised constitution, and 3 announcements were published for the Society.

The volume contains 2 portraits, 151 illustrations for scientific articles and notes, and 251 tables. Authors have been charged for illustrations exceeding 2 pages and for tables exceeding 33 per cent of the length of a paper. Divisions have been charged for the divisional abstracts of members for whom the Society published two abstracts presented at the annual meeting.

On December 1, 1947, there were 64 papers on hand; 35 had been accepted for publication and 28 of these were in press; 13 were being revised by the authors; and 16 were being reviewed by the editors. Between December 1, 1946 and December 1, 1947, eleven papers were withdrawn or rejected.

Transference of responsibility for reprint orders from The Science Press to The American Phytopathological Society delayed slightly the publication of some papers that might otherwise have been scheduled for the November and December issues. The necessity for price quotations on special forms and delay of the issuance of requisitions and purchase orders may account for a delay in publication of an article.

Report of Representative on Division of Biology and Agriculture, National Research Council, 1947. The activity of the Division of particular concern to our Society at the annual meeting in April, 1946 had to do with the proposed American Institute of Biological Societies. The proposed Constitution and By-Laws passed by the Organizing Committee, called together by the Division, were discussed and approved for reference back to the individual societies for consideration. Copies of the Constitution and By-Laws were distributed to members of the Society previous to the Chicago meeting. A discussion of these documents was submitted by your representative to the Council.

Report of the Necrology Committee. The following members died during 1947: A. E. DAVEY, ALBERT ISAKSSON, and RALPH JUSTO-PRATS.

Report of the Manager of Phytopathological Classics. Report for the fiscal year beginning October 1, 1946 and ending September 30, 1947:

Classic No. 1:	On hand, October 1, 1946	0	
Classic No. 2:	On hand, October 1, 1946	178	
	Sold during year	12	$ 6.00
	On hand, September 30, 1947	166	
Classic No. 3:	On hand, October 1, 1946	265	
	Sold during year	12	6.00
	On hand, September 30, 1947	253	
Classic No. 4:	On hand, October 1, 1946	328	
	Sold during year	11	8.25
	On hand, September 30, 1947	317	
Classic No. 5:	On hand, October 1, 1946	561	
	Sold during year	14	17.50
	On hand, September 30, 1947	547	
Classic No. 6:	On hand, October 1, 1946	646	
	Sold during year	13	9.75
	On hand, September 30, 1947	633	
Classic No. 7:	On hand, October 1, 1946	664	
	Sold during year	13	9.75
	On hand, September 30, 1947	651	

	$ 57.25
Value of books sent out (fiscal year 1946–1947)	$ 57.25
Money received as advance on Classic No. 8	1.00
Money received on orders of previous year	7.61
	$ 65.86
Money received during fiscal year 1946–1947	$ 61.36
Due on account	4.50
	$ 65.86

Assets:

Cash balance on hand, October 1, 1946 …….. . . …... . . .. ……… …	$642.28
Receipts during year …….. ………... ………… .. . ….. … ..	61.36
	$703.64

Liabilities:

Stamps ……… ………………………… ….. . ………….. . ……… .. . ….. .. . ….. ……	$ 5.00
Express charges ……… ……… … . ……….. .. . ……… .. … . . ….. ..	3.39
	$ 8.39

Balance on hand, September 30, 1947 . ……………… …… …………………………… .. .. …………	$695.25
Total due on account, September 30, 1947 ………… .. . ……… . . …… …..	$ 9.75

Report of the Editor of Phytopathological Classics. Number 8 of PHYTO–
PATHOLOGICAL CLASSICS will be off the press by year's end. Final page proofs
are now in process of correction. Number 8, containing M. J. Berkeley's ''Observations,
Botanical and Physiological on the Potato Murrain'' and selections from his ''Vegetable
Pathology,'' was prepared by a committee of the British Mycological Society, with
Dr. John Ramsbottom contributing a 3500-word illustrated biography. The price of
the 100-odd-page publication has been set at $1.25. All expenses incident to the new
volume will be paid out of proceeds from the sale of previous issues, thereby continuing
the policy of keeping PHYTOPATHOLOGICAL CLASSICS on a self-sufficient basis.
    Editing of the Berkeley manuscript entailed much labor, especially in comparing
the galley proofs with the originals. Petition for funds to engage an assistant was made
to Michigan State College's All-College Research Fund in the amount of $30.00, and the
petition was generously granted to the extent of $50.00. I suggest the Council write
Professor L. C. Emmons, Dean of the School of Science and Arts, Michigan State
College, East Lansing, Michigan, thanking him for his influence in arranging the grant.
Copy of such action to me would also be appreciated.
    Plans for future issues of PHYTOPATHOLOGICAL CLASSICS are under way,
and Tozetti's ALIMURGIA appears to be a likely subject for CLASSIC NO. 9.
Tozetti's writings have already been reviewed by Doctors P. P. Pirone and J. C.
Walker, both of whom deem the selection an interesting and worth-while treatise for
inclusion in the series.
    In discussing future plans, however, there is one proviso I should like to suggest,
and that is that any subsequent publishing be postponed until printing costs have
stopped spiralling. The per unit cost of PHYTOPATHOLOGICAL CLASSICS would
soon preclude sales, and I am not willing to see the 15-year-old record of solvency lost.

Report of the Placement Committee. During 1947, 54 plant pathologists had
applications filed with the Placement Committee. Prospective employers listed 63
positions with the Committee. A total of 214 individual applications were sent to
employers. Incomplete reports indicate that at least 5 plant pathologists obtained
positions through the efforts of this Committee.

Report of the Public Relations Committee for 1947. The procedure in publicizing
plant disease research has continued as in the past with the exception that ''IDEAS''
for popular articles have usually been routed directly to editors of the most suitable
journals instead of using a wider, impersonal distribution. Nineteen ''IDEAS'' were
released, of which 16 resulted in published articles in ''Farm Journal,'' ''Country
Gentleman,'' ''Better Homes and Gardens,'' ''Scientific Monthly,'' ''Farmer-Stock-
man,'' and other periodicals. In several instances the Committee has been of service
to the fungicide industry in providing information on preventable crop losses and
markets for fungicides. The Chairman of the Committee has been appointed to repre-
sent the Society in providing editorial assistance for ''Scientific Monthly.'' An exhibit
of the Committee's work was on display at the Chicago meeting. The expenses of the
Committee, as in the past, have been paid from remuneration received by individual
writers for magazine contributions.

**Report of the Committee on Biological Abstracts and Union of Biological
Societies.** The coverage of biological journals by Biological Abstracts has been rapidly
increased during 1946 and 1947 to include as many as possible of the journals which
could not be covered during the war and new journals published in Europe and Asia.
Up to July 1, 1947, over 850 assignments covering these journals had been made in
the preceding 18 months. The total journals being abstracted now number about 2800.
Because of the continued unavailability of many foreign publications, the abstracting
of a large share of these journals is being carried out by collaborators in the country

of origin of the journal. The Japanese journals are the only ones still unavailable for abstracting.

The increased cost of printing and binding has put a serious strain on the finances of Biological Abstracts. In 1946 it was necessary to withdraw $1320 from reserves. In 1947, with no visible large increase in revenues, the printing and mailing estimates alone have been increased $13,000 over 1946. A concerted effort for further support will be necessary.

The situation is more serious since the recent delays in appearance of the numbers, due entirely to difficulties in the printing and binding of the material, do not make good advertising. Abstracting is being kept up-to-date.

During 1946 Section H—Abstracts of Human Biology—was added. Beginning with January 1948 a new section—Abstracts of Cereals and Cereal Products—will be added. The latter will contain abstracts otherwise scattered through other sections of Biological Abstracts.

Report of the Committee on Regulatory Work and Foreign Plant Diseases. This Committee has been relatively inactive. It was appointed at the suggestion of Dr. P. N. Annand, Chief of the Bureau of Entomology and Plant Quarantine, as a committee to give advice on request. Only one question has been submitted, and I hope that this was satisfactorily answered. The recommendation is that the Committee be continued and that the chairman ascertain its possible future need and usefulness.

Report of the Committee on International Cooperation. Several members of the Committee met in Washington and discussed policy and activities. Various members of the Committee also discussed matters with officials of the Office of Foreign Agricultural Relations. The chairman of the Committee, on an extensive trip through Mexico, Central and South America, discussed with many pathologists the question of interchange of information such as that previously proposed by the Committee. There was enthusiastic response with respect to the desirability of this interchange, but it was evident that it was impossible to get the desired information from many countries because of lack of pathologists, lack of organization, or lack of facilities for accumulating and summarizing the necessary information. This makes it seem possible that it would be better to start with a less ambitious program.

A modified proposal therefore is made with respect to the interchange of information.

It is proposed that the Committee on International Cooperation of the American Phytopathological Society collaborate with the Committee on Inter-American Scientific Publication (Harlow Shapley, chairman; Mrs. Christina Buechner, executive secretary). Steps already have been taken to have certain summaries regarding plant diseases published in Latin American journals. The objective of the Committee on I.A.S.P. at present is to facilitate the interchange of scientific information between countries of the Western Hemisphere. It is hoped, however, to expand the project into other countries as soon as improvements in the international situation warrant it. As this organization has a central office in Boston, with a full-time secretary, and is desirous of facilitating publication of scientific papers in general; and as they have at least a modest sum of money as a working fund, it is proposed that we collaborate with them as a first step in attaining the broader objectives envisaged by the Committee.

It is recommended that the Committee be continued.

Report of the Committee on Resolutions. Be it resolved that the American Phytopathological Society express its grateful appreciation to the following for their contributions to the success of the 39th annual meeting:

W. B. Allington, J. C. Carter, Glenn S. Pound, and M. A. Smith for so capably handling all arrangements pertaining to the banquet, entertainment, and smooth functioning of all sessions.

A. C. Hildebrand, J. E. Kuntz, and John E. Thomas for supervising registration.

W. D. Valleau and Stephen Diachun for arranging all papers into sessions.

K. Starr Chester for so ably handling all publicity before and during the meeting.

The management of Hotel Stevens, and especially John W. Bowman, Director of Conventions and Exhibits, for many courtesies extended the Society.

The Chicago Bar Association for splendid cooperation in providing banquet facilities.

John Strohm, Associate Editor of "Country Gentleman," for his most interesting and informative address on Russian agriculture.

The American Association for the Advancement of Science for providing meeting rooms, projection equipment, operators; and other courtesies.

Be it separately resolved that the Society express its appreciation to President A. J. Riker, Vice-President R. S. Kirby, and Secretary E. M. Johnson for invaluable services during their terms of office, and to other officers, representatives, councilors, and committee members for their efficient work in conducting various activities of the Society during the past year.

Elections and Appointments. A committee from the Council opened and counted the ballots, results of which were announced to the Society at the banquet the evening of December 29: R. S. Kirby, President; W. D. Valleau, Vice-President; S. J. P. Chilton, Councilor-at-large. Curtis May was appointed Secretary for 1948–1950.

The Council recommended and the Society approved the appointment of J. H. Jensen as Editor for a three-year term through 1950; J. D. Wilson, J. G. Harrar, K. W. Kreitlow and Donald Cation as Associate Editors for three years through 1950; L. J. Alexander, Advertising Manager of PHYTOPATHOLOGY for 1948; L. C. Knorr, Editor of PHYTOPATHOLOGICAL CLASSICS for 1948; L. J. Tyler, Business Manager of PHYTOPATHOLOGICAL CLASSICS for 1948; J. C. Walker, Representative to American Institute of Biological Science; H. A. Rodenhiser on A.A.A.S. Council for 1948–49; and Charles Chupp on the Board of Editors, American Journal of Botany for 1948–50.

One hundred and eight applicants were elected to membership in The American Phytopathological Society.

Reports of Officers, Representatives, and Standing Committees are published on the previous pages. According to Society action at the Philadelphia meeting, reports of Special and Temporary Committees are not to be published in the annual report. All Committee reports submitted were considered by the Council. Those recommended for approval by the Council were accepted by the Society.

The Society approved the following recommendations by the Council:

1. That the Membership List Committee be given permission to publish a membership list in 1948.

2. That the Special Committee on Plant Disease Prevention be made a standing committee.

3. That the Standing Committee on Regulatory Work and Foreign Plant Diseases be made a subcommittee of the Plant Disease Prevention Committee.

4. That the name of the Special Committee on National Security be changed to Subcommittee on the Utilization of Plant Pathologists and Facilities in National Emergencies, and that it be under the Plant Disease Prevention Committee.

5. That there be added to the Plant Disease Prevention Committee a Subcommittee on Seed and Plant Material Certification Standards.

6. That the Standing Committee on Necrology be discontinued and that its functions, including the preparation of biographical sketches of deceased members, be assigned to the Secretary.

7. That the Standing Committee on the Union of Biological Societies (Biological Abstracts) be discontinued and that its functions be taken over by the Society's representative to the American Institute of Biological Sciences.

8. That a Subcommittee on Virus Type Culture Collection be added to the Committee on Nomenclature and Classification of Plant Viruses.

9. That the Temporary Committee on Sustaining Associates be made a special committee.

10. That the Fungicide Committee consist of seven members.

11. That the Subcommittee on Methods of the Fungicide Committee be discontinued for the time being.

12. That there be added to the Fungicide Committee a Subcommittee on Annual Summary of Results on Newer Fungicides, to continue the work initiated by the Potomac Division in disseminating this information.

13. That there be added to the Fungicide Committee a Subcommittee on Fungicide Nomenclature whose function shall be to study the important question of common names of fungicides in cooperation with other interested committees from the chemical, entomological, and other societies and the United States Department of Agriculture, and that they be further authorized to select suitable common names and to preempt their use by such means as they deem advisable, and that such names shall be published in PHYTOPATHOLOGY when they are ready for adoption.

14. That there be formed a Special Committee on Nomenclature of Plant Pathogenic Bacteria.

15. That there be formed a Special Committee on Teaching of Plant Pathology.

16. That the invitation to join the American Institute of Biological Sciences be submitted to the members for a mail vote, in a reasonable time, and if approved the dues will be increased one dollar beginning in 1949.

17. That the Society continue to appropriate $100 to Biological Abstracts.

18. The adoption of the following resolution: Be it resolved that The American Phytopathological Society express its interest in the proposal of Dr. Paul Neergaard for the issuance of an International Herbarium of Pathogenic Fungi (fungi exsiccati) to be

initiated and carried on under the auspices of U.N.E.S.C.O. or similar international organization, and agree to join with the Mycological Society of America and the British Mycological Society in sponsoring the project. Suitable precautions should be taken to guard against dissemination of potentially dangerous parasites. It is understood no financial outlay is involved.

19. That the editors of PHYTOPATHOLOGY are authorized to publish sheets containing news, notes, announcements, comments, and short items of interest. The activity is not to cost over $300 in 1948.

20. The adoption of the following resolution:

Whereas many foreign plant pathogens not only have already come into the United States but also have caused enormous damage, and

Whereas there are many other plant pathogens which might cause similar devastating loss if they should enter and become established, The American Phytopathological Society resolves that:

(A) The United States needs to have a greatly strengthened plant disease quarantine service at ports of entry or in countries of origin. Many plants carry diseases which do not show on seed or dormant stock. Such material should be given competent inspection while growing. This inspection should meet the standards now applied to nursery stock in most states. This is particularly important since pathogens brought in by aeroplanes will arrive more promptly and usually in more virulent condition than organisms introduced by the older forms of transportation.

(B) The United States needs more critical information about the susceptibility of American timber species and crop plant varieties to foreign parasites that might be introduced.

(C) More effectively to meet these needs, it is recommended that the U. S. Department of Agriculture request the necessary additional appropriations and arrange with the Department of State for development of cooperative agreements with those foreign countries interested in safe plant exchange on a mutually beneficial basis. The activity should include:

(a) the establishment in the cooperating country of test or trap plantings of important American species and varieties of interest to either cooperating country, for which the reaction to foreign pests and diseases needs to be ascertained;

(b) periodic observation by competent American and/or foreign specialists for diseases; and

(c) for those found important, study of the means of dissemination of the causal organisms.

(D) Similarly, test planting of important species and varieties of the cooperating countries may be established in appropriate regions of the United States.

21. The formation of the North Central Division of The American Phytopathological Society, an area including the states of Missouri, Minnesota, Kansas, Nebraska, South Dakota, North Dakota, Iowa, Illinois, Kentucky, Wisconsin, Michigan, Ohio, and Indiana.

22. That the time and place of the next annual meeting shall be left to the discretion of the Program Committee consisting of the President, Vice-President, Secretary, and Editor-in-Chief, with the suggestion that the meeting be held in early December, in connection with other societies, in the longitude of Pittsburgh or farther east.

23. That the Special Committee on Publication Problems be discontinued.

24. That there be published in 1948, in PHYTOPATHOLOGY, a Directory of Fungicides consisting of a list of fungicides with chemical, trade, and common names (if available) with the name of the manufacturer, as shall be prepared by the Subcommittee on Fungicide Nomenclature.

GROVER H. BURNETT

January 31, 1890–June 15, 1946

Dr. Grover H. Burnett, a native of Idaho, was graduated from the University of Idaho, 1917, with the degree of Bachelor of Science. Entering World War I as a Private, he earned a Commission. He then spent eight years in educational work in agriculture in Idaho, Washington, and California. From 1927 to 1933 he was on the staff of the Botany and Plant Pathology Department of Washington State College, where he received the degree of Master of Science in 1929 and Doctor of Philosophy in 1932.

The years 1933 to 1936 he spent in white pine blister rust control in the Northwest, and in study of the nursery diseases of conifers in Montana, Colorado, and Nebraska, for the Bureaus of Entomology and Plant Quarantine, and Plant Industry, respectively. From that time until his death he was engaged as Farm Planner for the Soil Conservation Service in Colorado.

Doctor Burnett was a man of good judgment and pleasing personality, an excellent cooperator and universally liked. During his period at Washington State College he published a number of creditable papers on the viruses of the Solanaceae and of Phlox and Delphinium. He took particular pleasure in the out-of-door features of his work. He was cautious in interpretation of results, and outstanding for the conscientious character of his work.

Doctor Burnett was a life member of The American Phytopathological Society.

JOSEPH HUNTER GOODING, JR.

August 31, 1891–April 14, 1947

J. Hunter Gooding, Jr., was graduated from Oregon State College in 1914 with the Bachelor of Science degree in Agronomy. He took advance courses at Columbia University, New York; New York University, and the University of Pennsylvania.

Except for two intervals as farm manager and orchardist, Mr. Gooding's entire career was with E. I. du Pont de Nemours and Company. In his 32 years with the company he rose from a clerkship at the Carney's Point, New Jersey plant to sales manager of the Semesan Division of the Grasselli Chemicals Department.

It was in 1923 that Mr. Gooding began his career in the field of seed disinfectants. By the time of his death he was recognized as one of the men who had made valuable contributions toward establishing the need for seed disinfection as common practice in the minds of the American farmer.

Mr. Gooding was a member of the American Phytopathological Society, The Grange, and other organizations.

Mr. Gooding has been described as being square in his dealings with coworkers and competitors alike, competent, cheerful, optimistic, and sociable. He was a fine example of the kind of person it takes for successful working relations between science and industry.

ROBERT ALMER HARPER

January 21, 1862–May 12, 1946

Robert Almer Harper was born at Le Claire, Iowa. He graduated from Oberlin College with the degree of Bachelor of Arts in 1886 and carried on graduate studies at Johns Hopkins University during 1888–89. He returned to Oberlin to receive the degree of Master of Arts in 1891. Following this he studied for several years at Bonn under the great botanist, Eduard Strasburger, receiving the doctorate from the University of Bonn in 1896.

Professor Harper's long and distinguished career as a teacher began when he went to Gates College as instructor of classical languages from 1886 to 1888. From 1889 to 1898 he was instructor and professor of botany and geology at Lake Forest College, Lake Forest, Illinois. From 1898 to 1911 he was professor and head of the Department of Botany at the University of Wisconsin. In 1911 Dr. Harper went to Columbia University as Torrey Professor of Botany and executive officer of the department. He withdrew from formal teaching in 1930 with the title of Emeritus Professor, but continued active in research and advisory work with graduate students at Columbia until 1937, when he moved to his farm near Bedford, Virginia, where his remaining years were spent.

Dr. Harper was active in the National Academy of Sciences, American Association for the Advancement of Science (vice-president for the plant sciences section in 1910), Botanical Society of America (president in 1916), Torrey Botanical Club (president 1914–16), American Phytopathological Society, Ecological Society of America, Society of American Naturalists, Philosophical Society, American Academy, and was a corresponding member of the Deutsche Botanische Gesellschaft.

In Professor Harper the qualities of a great, inspiring teacher and a penetrating, painstaking research worker were perfectly combined. His earlier works on the cytological details of reproduction in the powdery mildews, in Peziza, Pyronema, Hypochnus, and several rusts have long been considered classics in the field. Later his interest turned to problems of the fundamental structure and morphogenesis of living things. His presidential address before the Botanical Society in 1916 was an exceedingly penetrating consideration of the structure of protoplasm. His later papers deal with the factors involved in the development of form. Professor Harper's grasp of the structure and interrelationships of living things and his insight into fundamental biological problems were unparalleled.

JOHN LEONARD RUE

July 15, 1900–February 9, 1946

John Leonard Rue was educated in the public schools at Freehold, New Jersey, after which he studied at Stevens Institute of Technology, Hoboken, New Jersey.

Mr. Rue was employed by the Bureau of Plant Industry, U. S. Department of Agriculture, in 1927, to assist at the Bureau's Peach Disease Field Laboratory, Fort Valley, Georgia. He was appointed Scientific Aid in 1931, advanced through grades, and was promoted to Chief Scientific Aid in 1941.

During his employment at Fort Valley, he assisted in planning laboratory buildings and structures, maintained experimental nurseries, orchards, and greenhouses, and participated in investigations on the phony peach disease, peach rosette, winter injury, prolonged dormancy of the peach, and other research activities. In 1938 he was transferred to a new laboratory being established at Brownwood, Texas, by the Division of Fruit and Vegetable Crops and Diseases, for investigations on peach mosaic and other virus diseases of deciduous fruit trees, in which work he remained until his death.

Mr. Rue was a most loyal and dependable worker. He developed investigative ability, became proficient in virus research techniques, and was coauthor of several technical publications. His straightforward character and excellent personality endeared him to his associates and greatly furthered the cooperative work in which he was engaged.

GORDON ALEXANDER SCOTT

May 7, 1895–February 28, 1946

Gordon Alexander Scott, after four years' military service in World War I, graduated from the Ontario Agricultural College, Guelph (University of Toronto) in 1923 with the degree of Bachelor of Science in Agriculture. He received the degree of Master of Science from Macdonald College, St. Anne de Bellevue, Quebec (McGill University), in 1924 where he held the W. C. Macdonald scholarship in 1923 and the Chilean Nitrate scholarship in 1924.

In February, 1925, Mr. Scott was appointed to the Division of Botany and Plant Pathology and was stationed at the Dominion Laboratory of Plant Pathology, Saskatoon, Sask., under the late Professor W. P. Fraser. He resigned in November, 1929, to farm the old homestead in Ontario. He returned to the Division of Botany and Plant Pathology and was stationed at Ottawa, where he worked as a Plant Disease Investigator for the summers of 1932, 1933, and 1934. Mr. Scott served successively as Junior Plant Pathologist, 1934 to 1937, Assistant Plant Pathologist, 1937 to 1941, Agricultural Scientist, Grade 2, 1941 to 1945, Agricultural Scientist, Grade 3, October through December, 1945, and Agricultural Scientist, Grade 4 from January 1, 1946 to the time of his death.

"Mickey," as he was familiarly known to his associates and to the seed growers throughout Canada, was chiefly responsible for devising and developing examination methods for seed-borne disease. He worked wholeheartedly with the Cooperative Vegetable and Cereal Seed Treatment Committees of the American Phytopathological Society in testing the newer fungicides on a variety of seed crops. Mr. Scott was keenly interested in the improvement of seed-treating machinery and its role in the production of healthy seed. He successfully urged the Canadian Seed Growers Association to adopt measures to ensure disease examination for all Foundation and Elite as well as certain Registered seed stock grown in Canada. His outstanding personal qualities enabled him to obtain team work not only among his colleagues in research but also in the applied field among the farmers and those engaged in the seed trade. He will be greatly missed by his many friends and associates for his genial smile and his frank ways.

FUSARIUM DISEASES OF BROAD BEAN. II. FURTHER STUDIES ON BROAD BEAN WILT CAUSED BY FUSARIUM AVENACEUM VAR. FABAE [1]

T. F. YU AND C. T. FANG [2]

In an earlier paper (29) on wilt disease of broad bean caused by *Fusarium avenaceum* var. *fabae*, the symptoms of the disease and the morphological and cultural characters of the causal fungus were described. Since then investigation on the physiology, mode of infection and life history of the fungus, pathologic histology, and factors influencing the development of the disease were made and the results are herein presented.

THE FUNGUS

Isolates of *Fusarium avenaceum* obtained from the diseased roots of broad bean, like those occurring on other crop plants (27), comprise physiological races which are morphologically identical but pathogenically different. Some of these races cause only foot-rot, others cause typical wilt, and still others may cause either a foot-rot or sometimes wilt, dependent largely on environmental conditions. The results of a comparative study on the morphology, physiology, and pathogenicity of these races will be presented in a later report. In the present investigation, isolate No. 607–R–1, a typical wilt producing strain, was used throughout.

PHYSIOLOGY OF THE FUNGUS

Temperature in relation to the growth of the fungus. The relation of temperature to the growth of *Fusarium avenaceum* var. *fabae* was studied in Petri plate cultures of potato-dextrose agar which had been adjusted to pH 7. Four inoculated plates were placed in controlled incubators at each of the following temperatures: 20°, 22°, 24°, 26°, 28°, and 30° C. After the colonies were established the radial growth in each plate was recorded every 24 hours until the thallus covered the entire plate. The average daily growth was plotted for each of the other five temperatures (Fig. 1).

The fungus grew most rapidly at temperatures between 22° and 26° C., with the optimum at 24°–26° C. At 20° and 28° C., the rate of growth was greatly decreased. In another experiment, the fungus was incubated at both higher and lower temperatures. Almost no growth was detected at the higher temperatures tested (33°–35° C.) and very little at the lowest (5°–6° C.).

Hydrogen-ion concentration in relation to the growth of the fungus. Potato-dextrose agar was adjusted by the colorimetric method to the fol-

lowing hydrogen-ion concentrations: 4.4, 5.2, 5.8, 6.2, 6.7, 7.1, 7.6, and 8.1.
Transfers of the fungus were made to the centers of a series of Petri dishes
each containing 15 cc. of the medium and the plates, in triplicate, were in-
cubated at 26° C. The diameters of the colonies were measured every 24
hours. The results of the experiment, presented graphically in figure 2,
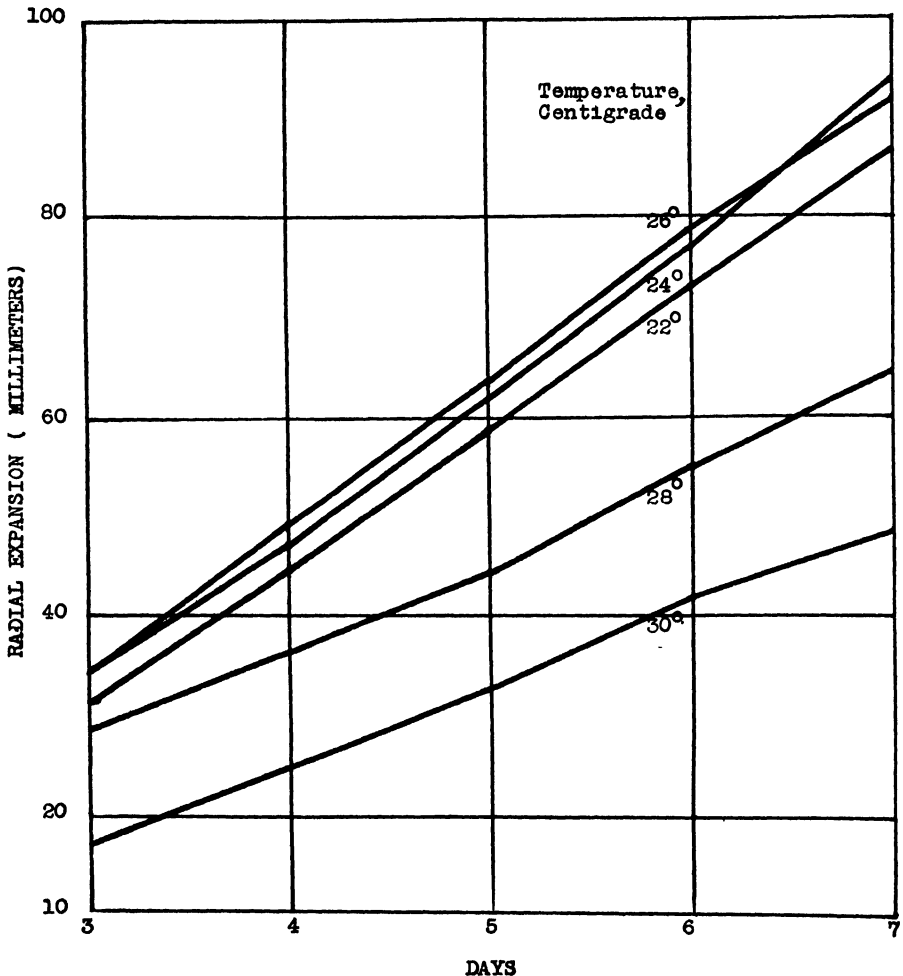
FIG. 1. Daily radial expansion of *Fusarium avenaceum* var. *fabae* on potato-dextrose
agar plates incubated at various temperatures.

indicate that the fungus grew in a wide range of pH concentrations, from
4.4 to 8.9 with the optimum between pH 5.8 and pH 6.7. The fungus
grew vigorously on the acid media and the growth rate fell rapidly when
media of greater alkalinity were used. The fungus tends to increase the
acidity of a medium on which it grows.

Spore germination. In moist Petri dishes containing hanging drops
of spore suspension, spores germinated most rapidly at temperatures near

the optimum for growth of the mycelium. At those temperatures almost 100 per cent of the spores germinated after 48 hours; while at 10° C. only 30 per cent or less had germinated. At temperatures above 30° C. the percentage of germination decreased. Spores germinated equally well in light or in darkness.

Sporulation. The relation of temperature to the frequency and abundance of sporulation of the fungus was determined both macroscopically and microscopically on agar slants incubated at various temperatures. The results of the test indicate that pionnotes and sporodochia were produced abundantly at temperatures from 10° to 28° C. with the optimum
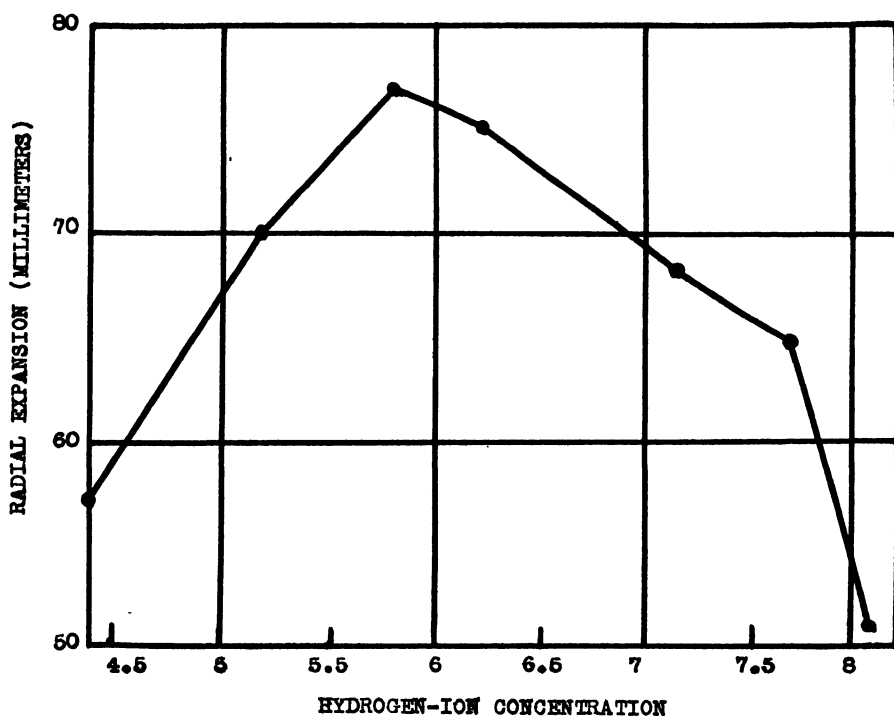
FIG. 2. Average diameters of 8-day-old plate cultures of *Fusarium avenaceum* var. *fabae* on potato-dextrose agar of various hydrogen-ion concentrations.

near 20° C. When spores were transferred to agar slants kept at 18°–20° C., abundant sporulation with slight mycelial growth appeared from 5 to 7 days after the transfer. In general, temperatures slightly lower than those for optimum growth of mycelium were favorable for sporulation.

ENVIRONMENTAL FACTORS IN RELATION TO THE DEVELOPMENT OF THE DISEASE

Soil moisture. Soil moisture is one of the important factors that may influence the development of soil-borne diseases. Linford (17) found that soil moisture had less influence than soil temperature upon pea wilt caused

by *Fusarium oxysporum*. Nevertheless, he found that wet soil slightly favored early development of disease symptoms but drier soil induced more rapid death of affected plants. Starr (21) reported that Alaska pea developed more wilt with an increase of soil moisture. According to Virgin and Walker (28), there was little difference in the rate of wilting caused by the near-wilt organism in dry and medium-moist soils when susceptible varieties of peas were tested. On the other hand, wilting was most rapid in medium-moist soil if resistant varieties were used.

Soil moisture studies were conducted in the greenhouse at an air temperature of 20°–26° C. Twelve-inch flower pots, coated and sealed at the bottom with paraffin, were filled with equal amounts of air-dried soils, the water-holding capacities of which had been determined previously. The soils were arbitrarily designated as dry, medium-moist, and wet, according to their moisture content. Surface-disinfected seed was planted at the rate of 5 seeds per pot. Each day the pots were brought up to the desired moisture content by first weighing them and then adding the necessary amount of water. For the first 7 days, heavy spore suspensions instead of pure water were added to the soils. In addition to these three groups of soil, certain pots of soil were inoculated with spore suspension, then kept saturated by flooding water over the soil surface to a depth of about 1.5 inches. Percentages of water-holding capacity for each soil group and percentages of wilted plants are given in table 1.

TABLE 1.—*The relation of soil moisture to the development of wilt in broad bean*

Condition of soil[a]	Water-holding capacity of soil in per cent	Total no. of plants	No. of wilted plants	Percentage of wilted plants
Dry	18–27	94	68	72.3
Medium-moist	44–65	91	71	78.0
Wet	70–89	95	12	12.6
Saturated	100	96	3	3.1

[a] Each group consisted of 10 pots.

The percentages of wilted plants in the wet and saturated soils were much lower than those in the other two soil groups (Table 1). The percentage of wilted plants in medium-moist soil was slightly higher than that in dry soil. This difference, however, was very slight and statistically insignificant. It was noted that plants growing in dry soil were so poor and stunted it was sometimes difficult to distinguish a wilt from the natural yellowing and drying that often occur with drought. The fact that the lowest percentage of wilted plants occurred in saturated soil confirmed results of the field surveys showing that there was less wilt in low fields where the soil remained saturated throughout the year. The reason for this is still unknown. It is probable that the vigorous growth of the plants in saturated soil may increase the disease tolerance of broad beans. The

results of the experiment, which was not an extensive one, show that broad bean developed less wilt with an increase of soil moisture.

Soil temperature. Soil temperature is by far the most important factor limiting the development and severity of wilt disease caused by species of *Fusarium* in crop plants such as cabbage (9, 24, 26), flax (14, 25), bean (2), tomato (4, 5), tobacco (12), pea (13, 28), potato (10), and watermelon (18, 19, 23).

Experiments for determining the soil temperature in relation to the development of wilt in broad bean have not been made. Field surveys, however, indicated that soil temperature is an important factor in limiting the distribution of the disease. Wilt, although occurring throughout the bean growing sections, was more destructive and prevalent in the northern part of Yunnan Province. In the southern part, where soil temperature was high, the disease was never destructive. The soil temperatures of eastern and western parts which were in the same latitude usually varied but little and the prevalence of the disease seldom varied. It seemed, therefore, that the distribution of wilt disease of broad bean in Yunnan was largely due to a difference of soil temperature.

Soil fertility. The application of chemicals and fertilizers to the soil may at times lessen the effect of certain root diseases although this procedure has seldom been used successfully on an extensive scale. Hoffer and Carr (11) have shown the pronounced influence of soil fertility on the development of root-rot of corn. Linford (17) stated that soil naturally rich in organic matter appeared more favorable for the development of pea wilt than poorer soils.

Although field surveys did not indicate any significant relation between soil type and the prevalence of wilt, it seemed that poor soils were more favorable for severe development of the disease than fertile soils. Experiments, therefore, were conducted to determine the influence of fertilizer on the development of wilt in broad beans. The tests were made on a very poor soil, using ammonium sulfate at the rates of 40, 60, and 120 pounds per Chinese mow[3]. Each plot consisted of 5 rows, 24 feet long and 2.5 feet apart. Seventy-five surface-disinfected seeds were sown in each row. The plots were replicated four times and arranged at random. Pure cultures of the fungus grown on sterilized barley seed were inoculated into the soil at the time of planting. The last records were taken at the end of February when the average percentages of wilted plants of the three treatments were respectively 62, 65, and 29, with the lowest readings occurring on the plots receiving the largest quantity of fertilizer. No further record was made after this time because of the appearance of foot-rot in most of the plots. The difference in percentages of wilted plants between plots to which 40 and 60 pounds of fertilizers per mow had been applied was statistically not significant. The plots receiving 120 pounds of fertilizer per mow produced the lowest percentage of wilted plants and the most vigorous

[3] A Chinese mow is equivalent to 1/6 acre.

plants. This again indicated that the vigorous growth of plants may possibly delay the development of the disease.

Soil reactions. Starr (21) found that the greatest number of pea plants wilted by *Fusarium oxysporum* appeared in soils with pH of 8.5. Wilting occurred over the entire alkaline range and none was found at pH 5.07 or less.

In preparation of soils with different pH values, sulfuric acid and sodium hydroxide were used in varying quantities and thoroughly mixed with soil consisting of one part sandy loam to three parts clay. The soil was allowed to stand for 10 days, in plots which had been previously sealed at the bottom with paraffin, and it was watered plentifully in the meantime. At the end of this period, the hydrogen-ion concentration of the soils was tested with a potentiometer. After the seed was sown, heavy spore suspensions were inoculated into the soil at the rate of 200 cc. per pot. The results of this experiment are given in table 2.

TABLE 2.—*Effect of hydrogen-ion concentration of the soil upon the development of wilt in broad bean*

pH value of soil	Total no. of plants	No. of diseased plants	Percentage of diseased plants
5.02–5.44	93	19	20.4
6.25–6.67	138	79	57.2
6.83–7.09	174	59	33.9
7.76–8.12	101	44	43.5
8.52–8.87	78	11	14.1

The difference in the total number of plants for each pH range was due to the varying number of pots and not to the effect of soil reactions on emergence of seedlings. Bean seed germinated and plants grew vigorously over the entire pH range. Abundant wilted plants appeared in the interval between pH 6.25 to pH 7.09, with the largest number occurring between pH 6.25 to pH 6.67. The results indicate that soil with slight acidity is most favorable to the development of the disease. Since wilting occurred over the entire range of pH tested, the application of fertilizers to change the soil reaction would not be of much value.

Surveys of soil reaction were made on high and low bean lands heavily infested with the wilt organism in Kunming, China, and its vicinity. The soil reactions of 14 fields on high land were all slightly acid. The soils of 9 out of 22 fields on low land had either neutral or slightly alkaline reaction, and those of the remainder were slightly acid. It seems, therefore, that hydrogen-ion concentration of soil is not an important factor in limiting the development of wilt disease.

PATHOGENICITY

In a previous paper (29), it was reported that the fungus was unable to cause infection of common bean, cowpea, tomato, potato, wheat, corn,

oats, and carrot.   Further inoculation experiments were made from time to time with attempts to find other hosts of the organism.   Negative results were obtained with the following plants, including several legumes: rice, sweet potato, pepper, radish, soybean, hairy vetch, white sweet clover, white clover, and red clover.   It was found that the fungus occasionally attacks pea and common vetches and causes yellowing in the lower leaves.

<center>MODE OF INFECTION AND PATHOLOGIC HISTOLOGY</center>

Infection may occur through rootlets, main roots, and hypocotyl.   In the field, infection occurs chiefly through the rootlets.   The first symptom of the diseased root is the discoloration and shrivelling of rootlets: these frequently yield cultures of *Fusarium avenaceum,* many of which are of the typical wilt producing strain.   Infection appears to occur on any part of the main root although it takes a long time for the fungus to reach the inner part of the root through the epidermis (Fig. 3, A).   The organism is different from the typical foot-rotting strains which usually produce conspicuous cortical lesions and more rapid collapse of cortical tissues.

Sometimes lesions are found on hypocotyls of young seedlings.   In order to demonstrate that the fungus may infect the plants through hypocotyls, the following test was made.   Surface-disinfected seed was planted in flower pots containing steam-sterilized soil.   After the seedlings attained a height of about 3 inches, a layer of soil was removed and a mixture of melted paraffin and beeswax poured on the newly-exposed surface.   A layer of infested soil was then laid on the paraffin layer which placed the diseased soil in direct contact with the hypocotyl.   Seedlings were dug out at regular intervals for laboratory examination.   Isolations made from the disease lesions on the hypocotyl yielded typical cultures of the fungus.

Microscopic examination of an infected root indicated that the most significant development of the fungus occurred in the vascular tissues, especially the lignified xylem vessels (Fig. 3, B).   The fungus rapidly grew upward along the tracheae to the stem.   In the meantime, it penetrated the thick-walled xylem and invaded other vascular tissues.   Certain xylem vessels contained granular gumlike deposits (Fig. 3, B, C, and D).   After the plant had completely wilted, mycelia of the fungus were found in the cortex region.   The distribution of the fungus in the stem tissues was the same as that in the root.   Abundant mycelia were first found in the lignified xylem vessels (.Fig. 3, C).   The fungus then penetrated the xylem wall to the cortex and from there invaded cambium and phloem (Fig. 3, D).   Only in the latest development of the disease were mycelia found sparingly in tissues outside the endodermis.   The fungus always grew upward and, in the later stage of the disease, usually extended through two-thirds of the stem.   In no instance, however, could the fungus be isolated from the flowering pedicels.

<center>LONGEVITY IN SOIL</center>

Wilt producing species of *Fusarium* can usually live in soil for a considerable time.   Starr (21) reported pea wilt was very destructive in a

field in southern Minnesota that had not grown a crop of peas for the previous six years. Porter (18) found that *Fusarium niveum* can live for at least 16 years in the soil in the absence of watermelon plants.

Diseased roots of broad bean were buried below the soil surface in 12-inch flower pots partly sunken in the ground outside the laboratory. At
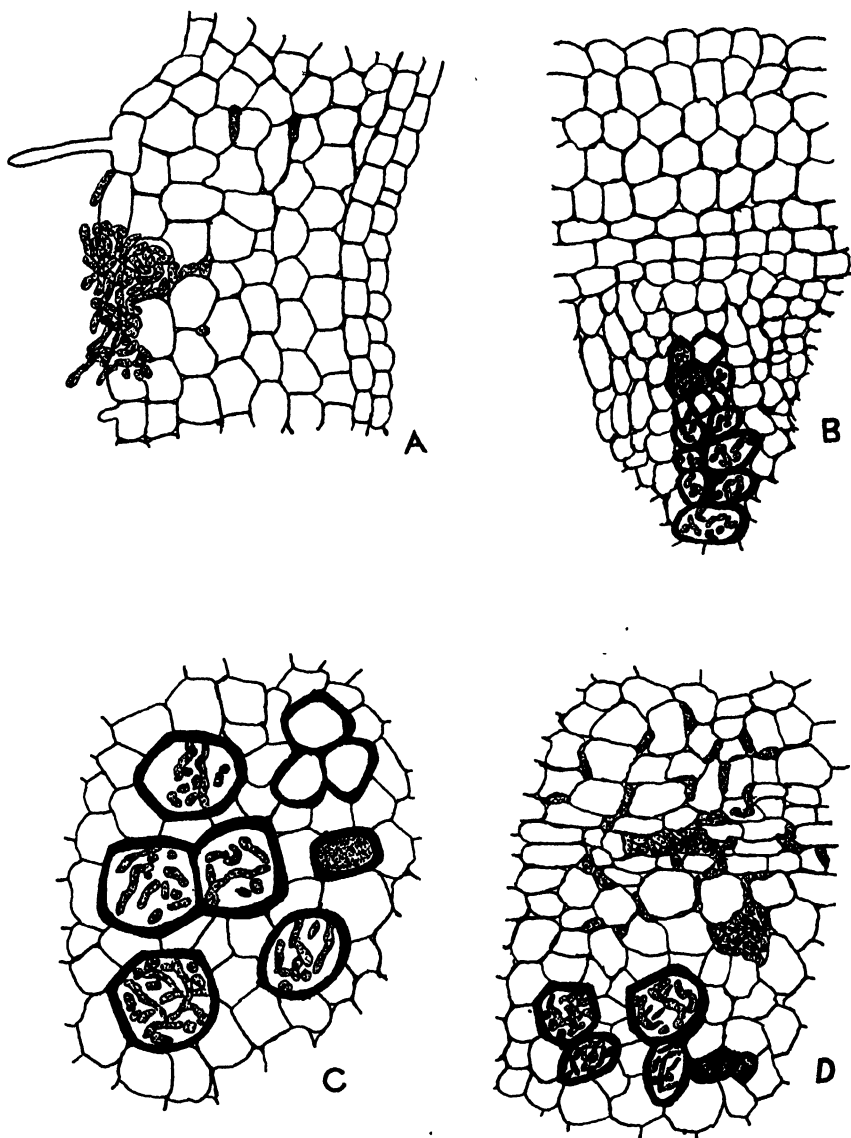
FIG. 3. Cross-sections of young root and stem of a diseased broad bean plant infected by *Fusarium avenaceum* var. *fabae*. A. Invasion of cortical region of the root by the fungus; B. Mycelium of the fungus in the xylem vessels of a root; C. Mycelium of the fungus in lignified xylem of the stem; and D. Invasion of lignified xylem, unlignified xylem, cambium, and phloem of the stem.

regular intervals, pots were removed to the greenhouse and planted with beans. The fungus remained viable for at least three years under these experimental conditions as indicated by the production of wilted plants.

OVERWINTERING STUDIES

Fungi causing root-rot and wilt frequently overwinter on old stubble and other debris in the soil. The experiment on the longevity in soil of *Fusarium avenaceum* var. *fabae* described above indicates that the fungus may live in the diseased roots throughout the winter. When cultures of the fungus grown on sterile wheat kernels were added to soil in flower pots and left outdoors, the fungus frequently sporulated in the spring on certain wheat kernels exposed on the soil surface. When these pots of infested soil were planted with broad bean seed, wilted plants were produced, showing that the fungus readily overwinters on debris in the soil or on its surface.

DISSEMINATION

Most of the wilt diseases caused by species of *Fusarium* have been found to be transmitted by seeds. Bolley (1) reported that flax wilt can be spread by way of seed on which spores of the fungus were lodged with dust at the time of threshing. Fulton and Winston (8) and Porter (18) have demonstrated the spread of watermelon wilt by diseased seeds. According to Edgerton and Moreland (5), spores of *Fusarium lycopersici* will remain on the surface of tomato seeds for about three months. Elliot and Crawford (7) showed that tomato seed naturally infected with *F. lycopersici* will carry the disease from field to field. The cotton-wilt fungus was also found to be seed-borne (6). In the case of pea wilt, Snyder (20) reported that it is sometimes transmitted by way of seed. Starr (21) found over 26 per cent of isolations from the seed coat of peas yielded the wilt organism, while no culture was obtained from cotyledons. Kadow and Jones (15) reported pea wilt fungus was carried to some extent by seed. On the other hand, Stokdyk (22) reported unsuccessful attempts to isolate *F. conglutinans* from cabbage seed and Kendrick (16) was unable to find the wilt organism *F. tracheiphilum* in cowpea seed. Linford also found no evidence of the wilt fungus being carried in pea seed.

In order to learn whether the broad bean wilt organism is seed-carried, pods were collected from wilted plants whose disease was induced by artificial inoculations of the plants with pure cultures of the fungus. The identity of the disease was confirmed by isolation experiments. Seeds were removed from the pods aseptically and stored in glass bottles. Isolations made from the seed failed to demonstrate the presence of the fungus either on the seed coat or in the cotyledons. On the other hand, it is interesting to note that, in another course of studies of the fungus flora of broad bean seed, cultures of *Fusarium avenaceum* var. *fabae* were obtained from the seed coat, but that isolations made from cotyledons failed to yield cultures of the fungus. Apparently, the fungus may be present on the coat of broad

bean seed as a result of contamination at the time of threshing. In harvesting, the entire bean plants including root systems are pulled out and carried to the farm yard where they are beaten by means of a swing-rod until all seeds are shed from the pods. Since *F. avenaceum* var. *fabae* sporulates freely on the basal stem of the diseased plant, this method of threshing offers ample opportunity for the seed to become contaminated with the spores of the fungus.

In Yunnan Province, broad bean is a winter crop and is rotated with rice. After the harvesting of beans in the first part of May, the rice fields are plowed and flooded in time for the transplanting of rice seedlings. As soon as the rice approaches maturity, the water is drained off and broad bean is sown between the rice plants. Since a rice field is flooded for about 4 to 5 months during the year, this led to investigating the question of how long the fungus would remain alive when submerged in water. Sterile barley seed on which the fungus had been grown was mixed with steam-sterilized soil in large Erlenmeyer flasks. These were incubated at 26° C. for one month, then flooded with a layer of sterile distilled water over the soil surface. At regular intervals, a portion of the soil was removed from the flask and placed on a potato-dextrose-agar plate. The results of this test showed that the fungus could live submerged for at least 12 months. It will be seen, therefore, that water drained from a rice field which has been infested with the wilt organism may serve as an important means of spreading the disease.

SUMMARY

1. *Fusarium avenaceum* comprises physiologic races which differ in pathogenicity by producing either foot-rot or wilt of broad bean. In this paper, results of investigation of a typical wilt producing strain designated as *F. avenaceum* var. *fabae* are reported.

2. Growth-temperature studies of the organism in Petri-dish cultures were made. The optimum for mycelial growth was 24°–26° C., the maximum below 33° C., and the minumum 5°–6° C.

3. The organism was capable of growing in media having a range of hydrogen-ion concentration from pH 4.5 to pH 8.9. Growth was most rapid between pH 5.8 and pH 6.7.

4. Spores germinated over a wide range of temperature and most rapidly when temperatures approached the optimum for mycelial growth. Temperatures around 20° C. were most favorable for sporulation of the fungus.

5. Dry and medium-moist soils were better for the development of the disease than wet and saturated soils. Medium-wet soil favored early development of symptoms; and dry soil, rapid death of affected plants.

6. No experiments were made to determine the influence of soil temperature upon broad bean wilt, although field surveys indicate that it is an important factor in limiting the distribution and severity of the disease.

7. The influence of soil reaction on wilt development was tested by growing broad bean plants in infested soils with a hydrogen-ion concentra-

tion varying from pH 5.02 to pH 8.87. The largest number of wilted plants occurred over a range of pII 6.25 to pH 6.67, although wilting might occur at any point over the entire range.

8. Fertilizers were found to have some influence on the development of wilt disease. More wilted plants were found in soils that had received the least quantity of ammonium sulfate. Poorer soils were more favorable than richer soils for the development of the disease.

9. Infection of the plant may occur through rootlet, main root, and hypocotyl. Of the three, rootlet infection is by far the most important in the field. The fungus invades chiefly the xylem of the root and stem, and from xylem spreads to cambium and phloem tissues. In the cortex, the fungus grew slowly and sparingly and it was only in the late stage of the disease that the fungus was found in the cortex of a diseased bean stem.

10. This fungus occasionally attacks peas and common vetches.

11. The fungus remained viable for at least three years in diseased roots buried under the soil surface. In the field it lives for a considerable time on old stubble and other debris in the soil.

12. No evidence has been found that the fungus was present inside the seed; but it was found on the seed coat, possibly by contamination at threshing time.

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PHOMA ROT OF GARDEN BEETS

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For several years a black rot of topped garden beets in storage has been one of the most important causes of loss of that commodity on the Chicago market during the winter months. Rail shipments received from Canada, New York, and Texas, as well as truck shipments of topped storage beets from Illinois and nearby States, have shown appreciable amounts of black rot on arrival at the wholesale market and in retail stores. Although this disease occurs commonly in topped beets that have been in storage for a few months, it has never been found in young bunch beets on the market. A survey of the literature shows that very little has been published on black rot in garden beets (4, 5, 7), although there is an abundant literature on a similar disease of sugar beets caused by *Phoma betae* (Oud.) Fr. (2, 3, 8). The present investigation was undertaken to determine whether or not black rot in garden beets is due to the same organism and to determine the degree of pathogenicity of the fungus for garden beets.

Storage beets on the market usually show black rot at the tip of the root (Fig. 1, A, B), although it occasionally occurs at the crown and in wounds on the side of the root. Because of the color of the beets, the small lesions are usually overlooked. On casual inspection the decay is seldom observed until the affected tissues become black and slightly sunken or until a flat, grayish white surface mycelium becomes evident. Internally the decayed areas are dark brown to black with a sharp line of demarcation between the healthy and diseased tissue. At first the invaded tissues are brown and water-soaked, but in the older lesions the affected tissues are black and somewhat granular, eventually becoming dry and spongy. With age, cavities lined with mycelium may be found within the spongy tissue of the larger lesions (Fig. 1, B). Although considerable grayish white mycelium develops on the surface of the older lesions under humid conditions no pycnidia have ever been observed on the beets.

Isolations made from the advancing edge of black-rot lesions in storage beets have consistently yielded a species of *Phoma*. Cultures of the organism obtained from garden beets grown in various localities appear to be identical. Furthermore, studies made of five isolates of the garden beet organism in comparison with authentic cultures of *Phoma betae* from sugar beet indicate that they all are the same species. On potato-dextrose agar (pH 7) the garden-beet organism produced abundant pycnidia that ranged from 210 to 560 μ in diameter. The conidia measured 4.3–8.1 × 2.9–5.8 μ, averaging 5.7 × 3.5 μ.

No significant differences were found in the morphological character-istics of the various isolates from garden beet and sugar beet lesions. The fungus causing black rot of garden beets is therefore considered to be identi-cal with *Phoma betae* (Oud.) Fr., the organism described as the cause of the sugar-beet black rot.

The cultures of *Phoma betae* isolated from garden beets grew on potato-dextrose agar (pH 7) through a temperature range of 35° to 95° F. Opti-mum growth was obtained at 75° F. The average diameters of colonies developed on potato-dextrose agar in 7 days at temperatures ranging from 35° to 95° F., in 10-degree intervals, are shown in figure 2.

FIG. 1. A. Natural lesion at the tip of the beet, showing surface mycelium. B. Longitudinal section of a beet with natural infection.

In order to establish the degree of pathogenicity of *Phoma betae* for garden beets, both whole and sliced beets were inoculated. In whole beets, V-shape punctures $\frac{1}{8}$ to $\frac{1}{4}$ inch deep were made with a sterile scalpel and a square of agar bearing mycelium and pycnidia was placed in the wound. In other experiments, wells about $\frac{1}{2}$ inch deep were made in whole beets with a sterile $\frac{1}{4}$-inch cork borer and the inoculum was placed in the bottom and the plug replaced.

In preliminary tests it was found that inoculated beets stored in moist sand remained in better general condition and developed more decay than those stored in moist chambers. Beets inoculated by the scalpel method and stored in moist sand for 6 weeks developed lesions averaging 5 mm. in diameter at 45°, 12 mm. at 55°, and 6 mm. at 65° F. Beets inoculated by the well method and stored at 50° for 4 weeks developed internal lesions ranging from 5 to 35 mm. in diameter. Both old storage beets and young bunch beets were inoculated in this fashion and in most instances greater decay developed in the older beets, thus indicating their greater suceptibility to invasion.

In further tests of pathogenicity, disease-free storage beets were cut aseptically into slices ½ inch thick and these were placed on Syracuse dishes in moist chambers with a small amount of water in the bottom of each chamber.   Inoculations were made by placing a small amount of agar bearing mycelium and pycnidia from a vigorously growing culture of *Phoma betae* on the center of each slice.   The moist chambers were held at 35°, 45°, 55°, 60°, and 65° F.   After one month some decay developed throughout this temperature range, but the greatest depth of penetration (10 mm.) occurred at 45° and 55°.   At these two temperatures, as well as the other three, the organism grew over the top of the slice to produce a flat gray
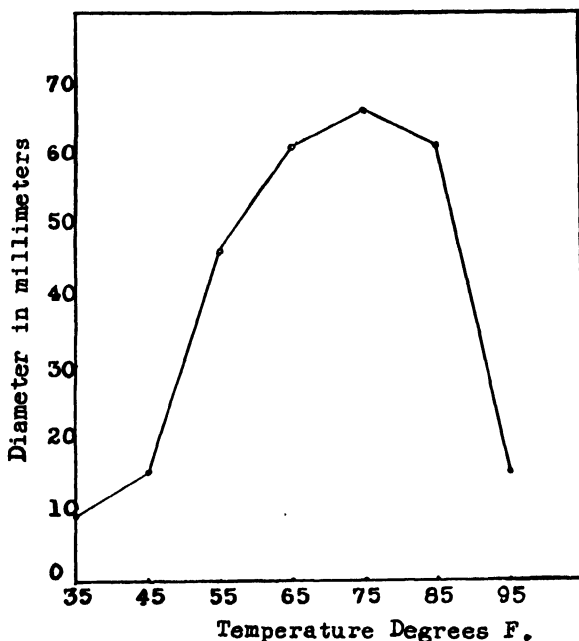
FIG. 2.   Growth of *Phoma betae* on potato-dextrose agar (pH 7) in seven days at various temperatures.

mycelium covering ¼ to ¾ of the surface.   In other inoculation tests, with slices of old storage beets in comparison with slices of young beets, it was found, as in the previous whole-root inoculations, that the rate of decay was slightly greater in the old beets.

Five different cultures of *Phoma betae* from garden beets and 3 from sugar beets were used in inoculation tests to determine whether there was any difference among them in pathogenicity.   Inoculated slices of storage beets were held at 50° F. for one month.   At the end of this period all slices showed some decay, but 2 sugar beet isolates of *P. betae* produced slightly more decay than the garden beet isolates.   Of the 5 garden beet isolates 2 caused more extensive decay than the other 3, thus indicating the possibility that there may be some difference in pathogenicity between strains of *P. betae.*   The organisms were recovered in each case by reisolation.

For inoculation studies with seedlings, seed of the Detroit Dark Red variety was planted in the greenhouse. When the seedlings had developed their foliage leaves they were transplanted to larger pots, three seedlings per pot. When the plants were about one month old two pots were sprayed with a water suspension of *Phoma* spores of a garden-beet isolate, one pot was covered for two days and the other was left uncovered. No spots were ever observed on the leaves of either pot. Two weeks later two more pots were inoculated in the same manner with negative results. One month later two more pots were inoculated; and in this lot, two days after inoculation, lesions were found on the older leaves of the plants in the pot that had been covered, but no infection occurred on the leaves of the plants that had not been covered. A sugar-beet isolate was also used to inoculate a pot of covered plants two months old, and similar leaf spots were produced within two days.

In each case infection occurred only on the older leaves (9 cm. long). The spots produced were small (2 to 3 mm. in diameter) with tan centers and red borders. The lesions did not enlarge much with age. The center of some of the older spots dropped out, producing a shot-hole effect. *Phoma betae* was reisolated from these leaf spots.

Pool and McKay (6) found that only mature sugar-beet leaves are infected with *Phoma betae* and then only through some lesions on the leaf's surface, such as insect injuries. They also found pycnidia developing in 10 days to 2 weeks on lesions 1 to 2 cm. in diameter. In the experiments reported here on garden beets, leaf spots developed on leaves that had not in any known way been injured mechanically. However, the growth of the lesion was halted in some way and no pycnidia developed on lesions one month old.

Repeated attempts to infect beet seedlings by inoculating the soil with water suspensions of spores, by burying the mycelium and pycnidia in the soil, or by burying rotten beets in the soil have failed, except in one case where a piece of inoculum was placed in the hole and a seedling was transplanted on it. These results indicate that the fungus is a weak pathogen and is not able to infect uninjured seedlings. Edson (2) considered *Phoma* capable of infecting beets only during periods of especial susceptibility, such as old age or low vitality.

Pool and McKay (6) list beet balls (seed), wind, irrigation water, insects, and dung as the agents of dissemination of *Phoma* spores in sugar beet fields. They considered the seed to be the most effective agent of dispersal, with wind and irrigation water playing secondary roles. Coons and Stewart (1) found *Phoma* present in carefully selected sugar beet seed.

Seeds of the Detroit Dark Red variety were bought from three commercial sources and germinated in moist cheesecloth. Over half of the seedlings were killed by *Phoma* in two of the lots. In the third lot about 20 per cent of the seedlings were infected. The blackened seedlings were examined microscopically and found to be covered with pycnidia of *Phoma betae*. The

fungus was isolated from the seedlings and was proved to be pathogenic for beets by inoculation tests.

In studies on the longevity of *Phoma betae* in garden-beet seed, Newton and Bosher (5) found that death of seedlings caused by *Phoma* decreased over a period of 5 years from 30 to 5 per cent. No decline in percentage of seed germination was noted.

Edson (2) showed that in sugar beets *Phoma* may live for a long time in inconspicuous lesions on seedlings and roots and eventually cause severe rot of the roots in storage. The studies here presented indicate that infection and decay of garden beets caused by *Phoma betae* probably occurs in the same manner as Phoma rot in sugar beets.

SUMMARY

A black rot of garden beets from storage is described as it occurs under market conditions. Comparison of the fungus isolated from garden beet lesions with *Phoma betae* from sugar beets indicates that they are the same species.

Optimum growth of *Phoma betae* from garden beet on potato-dextrose agar occurred at 75° F. with almost equal growth at 65° and 85°. Wound inoculations produced infection of both old and young garden-beet roots, but most severe decay occurred in the old roots.

Beet plants grown in the greenhouse and atomized, when about 2 months old, with a water suspension of spores of *Phoma betae* developed leaf spots in the older leaves; younger plants similarly inoculated were not infected.

In germination tests in the laboratory, with three lots of commercial garden beet seed, 20 to over 50 per cent of the seedlings were killed by this fungus.

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ANGULAR LEAF SPOT OF KUDZU CAUSED BY A NEW SPECIES OF MYCOSPHAERELLA[1]

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INTRODUCTION

In October, 1946, a leaf disease of kudzu (*Pueraria thunbergiana* Benth.) not hitherto observed was seen in a planting at Experiment, Georgia. The disease appeared to be responsible for the premature shedding of many leaves and varying degrees of injury to others. Observations were made in other kudzu plantings, and the disease was found at two additional locations, one a 20-year-old planting, also on the Experiment Station grounds, and the other a commercial planting 15 miles distant. In May, 1947, the same disease was found in several counties in central and south Georgia and in two locations in Alabama. Bain and Presley (1) reported having found the same leaf spot in Mississippi in the summer of 1946. The potential seriousness of this disease has made it worthy of further study. The results of an investigation into its nature and cause form the basis of this paper.

SYMPTOMATOLOGY

The disease seems to be limited to the leaves of the kudzu plant, leaves of all ages being susceptible. The lesions usually are angular, although some tend to be circular. Since the angular outline is the most characteristic feature of the lesion, the common name angular leaf spot is suggested (Fig. 1, A).

At first the lesions are evident only on the upper side of the leaf, but eventually they involve the entire thickness of the leaf and are visible on both sides. Young lesions are light brown, but they gradually darken to dark brown or almost black. They often have a yellowish margin or a slight yellowish halo. Old lesions may have dark gray centers with darker margins. The lesions, scattered in a miscellaneous manner over the leaf, are small at first but gradually enlarge, often merge, and eventually involve large areas or even the entire leaflet. In the field individual lesions most commonly vary from 1 to 10 mm. in diameter. The lesions may be more or less thickly covered with dark brown conidial tufts, which are abundant on the upper surface of the leaf, but sparse on the lower surface. When large areas of the leaflet have been killed, as when the lesions are

sufficiently numerous or when many of them have coalesced, the remaining tissue may turn yellow and finally brown, and the entire leaflet may fall off. Practically the entire upper surface of some of these dead leaflets may be covered with conidial tufts.

FIG. 1. A. Angular leaf spot on kudzu leaflets resulting from natural infection. Leaflet at the left was healthy. The other two leaflets illustrate the appearance of the lesions on the upper (center) and lower surfaces (right), respectively. × ½. B. Halo spot on kudzu leaflets resulting from natural infection. The leaflet at the left had lesions with a small necrotic center and large halo and that at the right had halo spot lesions with relatively large necrotic centers and small but very conspicuous halos. × ½.

So far as known, the only disease of kudzu that might be confused with angular leaf spot is halo spot, (*Pseudomonas phaseolicola* (Burk.) Dowson). In some stages of their development it is difficult to distinguish these two diseases, although in other stages they differ greatly. The halo

spot lesion commonly seen in nature during the early part of the growing season or during wet spells, has a small dead center surrounded by a rather large halo (2, 3). When the spots are numerous the halo is small (Fig. 1, B). When the halo is small and the necrotic center relatively large, halo spot is similar to young lesions of angular leaf spot. In well developed lesions of angular leaf spot, however, the halo is much less pronounced (Fig. 2, A); and if the humidity has been high, conidial tufts of the causal fungus may be seen scattered over the upper surface of the lesion.

Infected leaflets held in a moist chamber over night have bacterial ooze on the lower surface in the case of halo spot or conidial tufts on the upper surface in case of angular leaf spot.

ETIOLOGY

Taxonomy

The fungus associated with angular leaf spot of kudzu appears to be identical with *Cercospora puraricola* Yamamoto (8). A spermagonial and a perithecial stage have been found in the course of this study. Since the perithecia are spherical, short necked, embedded singly in the host leaf tissue, and have hyalodidymous ascospores and fasciculate, aparaphysate asci, this fungus should be placed in the genus *Mycosphaerella*. Since the perfect stage has not been reported previously, it is described as follows:

Mycosphaerella puraricola sp. nov.

Stat. Conid.: *Cercospora puraricola* Yamamoto. Trans. Sapporo Nat. Hist. Soc. 13(3): 142–143. 1934.

Perithecius plerumque epiphyllis, punctiformibus, in maculis- dispositis, nigris, innatis, sphaeroideis, 61–93 μ (medio 71.2 μ) diam., poro praeditis; ascis fasciculatis, aparaphysatis, clavatis, breve stipitatis, octosporis, 44.8–60.8 × 9.3–9.9 μ; ascosporis hyalinis vel pallide viridulis, 1-septatis, ad septum leniter constrictis, fusoideis, rectis vel inaequalibus, utrinque obtusiusculis, distichis, guttulatis, 14.3–22.2 × 2.7–4.5 μ (medio 19.7 × 4.2 μ).

Hab. in vere in foliis dejectis *Puerariae thunbergianae* Benth., Experiment, Georgia, U.S.A.

Spermagoniis nigris, punctiformibus, innatis, globosis, 42–67 μ (medio 52.7 μ) diam., poro praeditis, plerumque epiphyllis, cum stromatibus ascogonialibus in maculis aggregatis; spermatiis hyalinis, bacillaribus, plerumque 3 × 1 μ.

Hab. in autumno in foliis vivis atque dejectis *Puerariae thunbergianae*.

Status conidicus: Maculis 1–10 mm. diam., angulosis, brunneolis, dein nigro-brunneis, zonis flavidis circumdatis; conidiophoris plerumque epiphyllis, simplicibus, 1–6 septatis, non-constrictis, 20–84 × 4–4.5 μ, erectis vel sub-erectis, laxe fasciculatis, olivaceo-brunneis, erumpentibus, e stromate sphaerico, fusco, pseudo-parenchymatico, innato oriundis; conidiis hyalinis vel pallide olivaceis, cylindrico-obclavatis, rectis vel curvulis, 1–16 septatis, non-constrictis, vertice attenuatis, basi rotundatis vel truncatis, 25.2–126 × 3.0–5.3 μ (medio 69.7 × 4.3 μ).

Hab. in vera, aestate, atque autumno in foliis vivis *Puerariae thunbergianae*.

Type collection B.P.I. no. 71516, Experiment, Ga. May, 1947, Weimer and Luttrell.

Collections of both the perithecial and conidial stages have been deposited in the Mycological Collections of the Bureau of Plant Industry, the Farlow Herbarium, Harvard University, and the Royal Botanic Garden at Kew, Surrey, England.
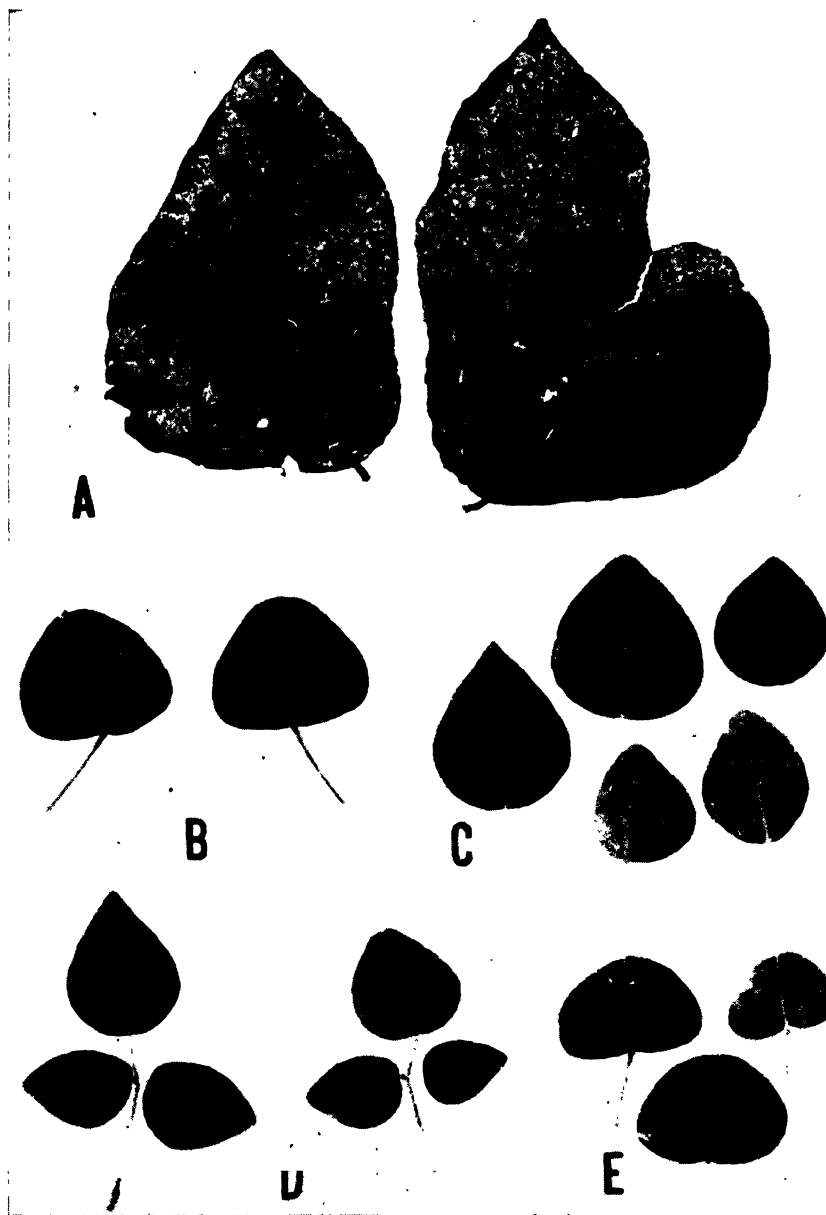
FIG. 2. A. Heavy natural infection of kudzu leaflets with angular leaf spot. Lesions have a relatively large amount of necrotic tissue and small halos. × ½. B. Two monofoliate kudzu seedling leaves with angular leaf spot lesions resulting from inoculation. Some lesions have a faint or conspicuous yellowish halo but the affected tissue is largely necrotic. Inoculated with a culture of the conidial stage on January 14, 1947, and photographed February 12, 1947. × 1⅓. C. Healthy leaf at left. Upper and lower leaves at right illustrate lesions on the upper and lower surfaces, respectively, 47 days after inoculation in the greenhouse. × ⅘. D. Two trifoliate leaves showing angular leaf spot lesions 66 days after inoculation with a conidial culture. × ⅖. E. Three monofoliate seedling leaves with lesions resulting from inoculation with a pure line asco-spore culture. These lesions are identical with those produced by the conidial culture. × 1.

Isolations and Inoculations

The *Cercospora* stage of the angular leaf spot fungus was isolated by placing infected leaves in a moist chamber for 24 or 48 hours and then transferring spores from the conidial tufts to agar by means of a sterile needle. Tissue plantings from leaves brought from the field often gave a *Colletotrichum* sp. that was acting as a secondary invader. The *Cercospora* grows slowly in culture and produces conidia sparsely on some media. Cultures of the ascospore stage used in these investigations were obtained by catching ascospores shot from pieces of the over-wintered leaves on agar and transferring single spores or groups of spores to agar tubes.

Since conidia from culture were not sufficiently abundant to be satisfactory for inoculation purposes, a combination of macerated hyphae and spores was used. The fungus was grown on a liquid medium made according to Leonian's (5) malt-agar formula with the agar omitted. The fungus colonies were removed from the culture medium, macerated in sterile tap water, and placed on the leaflets to be inoculated.

An inoculation experiment was set up on January 14, 1947, in which very young colonies of the fungus obtained from conidia and grown on Leonian's medium were used as inoculum. The plants inoculated were seedlings grown from seed collected locally, scarified by immersing in commercial sulphuric acid for one hour at 60° F. as described by Tabor (6), planted in sterilized soil in pots, and held in the greenhouse. The plants when inoculated were very small, usually having two monofoliate and one trifoliate leaf each. One or more pieces of inoculum were placed on each monofoliate leaf of 13 plants. Two plants in one pot were held as controls. After being inoculated, the plants were kept in a moist chamber for 96 hours at temperatures varying from 23° to 25° C. No leaf spot resulting from inoculation was positively identified until February 3, 1947, when a few spots from 1 to 2 mm. in diameter were evident on some leaves. These lesions slowly enlarged and additional ones became evident. On February 11, a total of 9 leaves on six inoculated plants had one or more lesions. There was no infection of the control plants. The lesions at first were yellowish to light brown, but gradually darkened to very dark brown, often with a yellowish border as illustrated by two monofoliate leaves shown in figure 2, B. Twenty-four of the 26 leaves inoculated had one or more typical lesions on February 26. The fungus was recovered from some of the infected leaves by placing them in a moist chamber for 24 hours and then picking off spores and transferring them to agar.

A second experiment was initiated on January 27, 1947, using the same methods except that the inoculum was placed on the under sides of the leaves where stomata are much more abundant. Leaves on nine seedlings were inoculated, and two seedlings were held as controls. The temperature in the moist chamber varied from 22° to 27° C. during the time the pots were held there. On February 11, there were no lesions that could

be recognized with certainty, but a few small spots were suspected of being immature lesions. By February 26 there were typical lesions on most of the leaves that had been inoculated. The lesions were sufficiently numerous and large to involve and kill considerable areas of some leaves (Fig. 2, C and D). The fungus was recovered from a number of lesions, and an abundance of spores formed on the lesions when held in a moist chamber for 24 hours. It is evident that in less than a month from the time of inoculation a large part of some leaves had been invaded and killed. For the most part the lesions ranged from 2 to 4 mm. in diameter, except when they had merged and involved larger areas. The earliest symptoms of infection were not apparent until about two weeks after inoculation. Infection can take place from either the upper or the under side of the leaf. Further inoculation experiments demonstrated that the fungus isolated from the lesions resulting from inoculation was equally as pathogenic as the original isolate.

Proof of the pathogenicity of the perfect stage of this fungus was obtained in an experiment initiated April 21, 1947. Young seedlings having only monofoliate leaves and grown from sterilized seed planted in sterilized soil were used. Three cultures were used in this experiment: (1) one of the original conidial cultures whose pathogenicity had been proved, (2) an isolate from a leaf infected in a previous experiment, and (3) a culture from a single ascospore. The inoculated plants were held in the moist chamber at temperatures of 25° to 28° C. for 96 hours. The first evidence of infection was noted on May 7, and by May 14 typical *Cercospora* lesions were present on some of the leaves inoculated with the ascospore culture. On May 19, nine plants inoculated with the ascospore culture had one or more typical lesions on one or both of the leaves inoculated, or a total of 14 out of 18 leaves were infected (Fig. 2, E). Seven of the 10 leaves on five plants inoculated with the orginal conidial isolate and five of the 12 leaves inoculated with the reisolate showed infection. There was no infection on any of the control plants. Thus 100 per cent of the plants inoculated had one or more leaves infected. Perhaps it should be explained that usually only two, rarely three, of the monofoliate leaves are produced by a seedling, hence only two leaves could be inoculated in the earliest seedling stage. The results of this experiment prove the pathogenicity of the ascospore stage and show that it produces lesions identical with those produced by the conidial stage under identical conditions. This constitutes proof of the connection of the conidial and perithecial stages of the fungus.

LIFE HISTORY

Isolates of the fungus grew slowly on all media employed, namely, oat meal, corn meal, Bacto nutrient, and Leonian's malt agars, Leonian's malt agar formula with the agar omitted, sterile oats, and kudzu stems. Of these media, Bacto nutrient agar was the least satisfactory. Leonian's

agar was slightly better than corn meal or oat meal agars. Growth of the fungus on agar was most rapid at 26°–28° C. No growth took place at 36° C., and it was very slow at 7°–8° C. The colonies were hemispherical in shape and olivaceous in color. When the growth was submerged, they were darker. The hyphae soon became olivaceous to dark brown and, under suitable conditions, formed conidia. The conidiophores were produced singly on the vegetative hyphae as simple branches growing at right angles to the parent hyphae (Fig. 3, C). Each conidiophore was divided by septations into three or four cells. Anastomoses between hyphae and be-
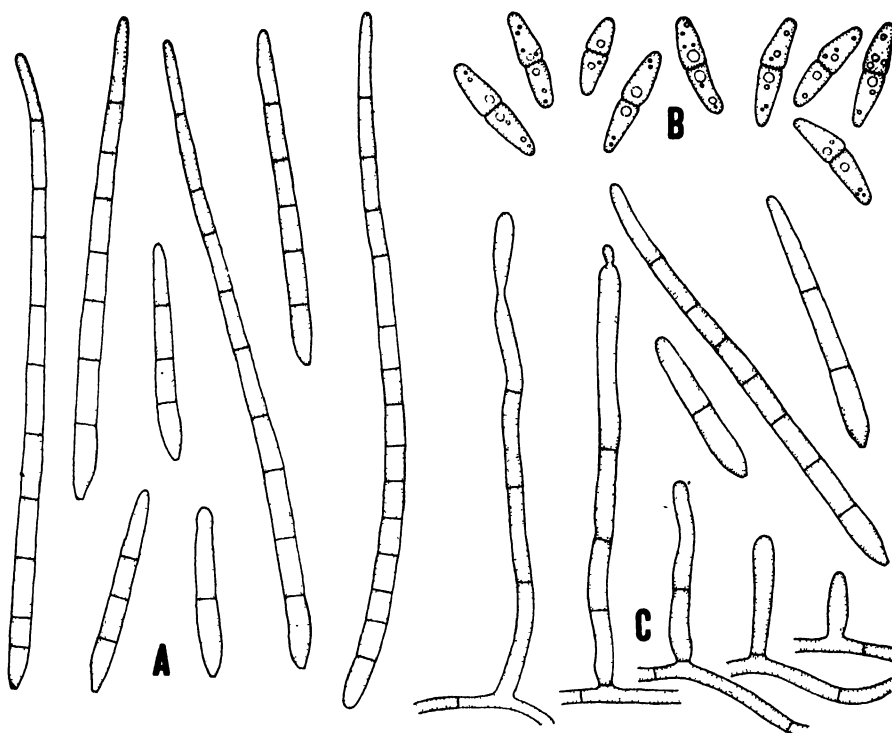
Fig. 3.   A. Conidia of *Mycosphaerella puераricola*.   × 750.   B. Ascospores of *M. puераricola*.   × 750.   C. Different stages in the development of conidia of *M. puераricola* in an ascospore culture.   × 750.

tween hyphae and conidiophores were frequent. The conidia were borne singly at the apices of the conidiophores and were identical with those produced in the field on kudzu leaves. Conidial production was not abundant on any medium.

The fact that isolates from ascospores were identical in appearance and growth response in culture to those obtained from conidia and that the mycelium of the former produced identical conidia in culture is additional proof that these spores are different stages of the same fungus.

Primary infection of kudzu leaves in the spring is accomplished by

either ascospores or conidia from overwintered leaves infected during the previous season. Study of cleared pieces of kudzu leaves showed that stomata are relatively rare on the upper surface. Since infection takes place readily when inoculum is placed on the upper surface of the leaf, it seems probable that the fungus is capable of direct penetration of the epidermis. The mycelium is both intracellular and, at least in late stages, intercellular. Inoculation experiments showed that the incubation period is approximately two weeks. At the end of this time the occupied tissue becomes necrotic, and the fungus fruits in the dead tissue of the resulting leaf spot. The conidial fructifications consist of erumpent clusters of densely crowded, erect or suberect conidiophores arising from globose, pseudoparenchymatous stromata embedded in the mesophyll. The conidiophores usually are simple, 1–6-septate (commonly 3–4-septate), non-constricted at the septa, olive-brown, somewhat lighter in color and slightly attenuated at the apex, and measure $20-84 \times 4-4.5\,\mu$. Often one or more old conidial scars are visible. The conidial tufts are formed on both surfaces of the leaf but occur more commonly on the upper surface.

Conidia are formed abundantly on the host during wet weather and on leaves held in a moist chamber for 24 hours. The conidia (Fig. 3, A) are almost hyaline to pale olive, straight or more commonly curved, cylindric-obclavate, truncate or rounded at the base, rounded at the tip, distinctly tapering toward the apex and slightly near the base, 1–16-septate, non-constricted at the septa, and measure $27.3-126 \times 3.2-5.3\,\mu$ (av. $72.7 \times 4.3\,\mu$). They are borne singly at the apices of the conidiophores. In the formation of a conidium the conidiophore produces a bud at the apex. This elongates and becomes septate to form the conidium. The developing conidium is marked off from the conidiophore by a constriction. At maturity it separates from the conidiophore at a cross wall laid down in this constriction. The truncate base of the conidium thus marks its point of attachment to the conidiophore.

The conidia produced on the host constitute a source of inoculum for repeated infections of new leaves throughout the growing season. The conidial stromata may overwinter on fallen leaves and produce conidia the following spring. The fungus, therefore, is capable of surviving from year to year in the conidial stage alone.

Spermogonia and perithecial initials develop within the lesions on moribund or dead leaves during the fall. Although they may be present on both surfaces of the leaf, they are much more abundant on the upper surface. In the earliest stages of their development they cannot be distinguished. Both structures originate from the internal mycelium as small stromata. They appear in the mesophyll just beneath the epidermis and usually are formed independently of the conidial stromata. The stromata enlarge until they raise the epidermis and finally become erumpent at the apex. The outer layers of cells are brown and thick-walled. Hyaline cells form a plectenchymatous or pseudoparenchymatous core. Although they

remain similar in external appearance, spermogonia and perithecia differ in further internal development. The hyaline cells in the interior of the developing spermogonium produce quantities of hyaline, bacilliform spermatia measuring approximately $3 \times 1 \mu$. As the spermatia are formed the cells that produced them disintegrate, leaving the spermogonium at maturity composed of a thin wall, one or two cells thick, enclosing a mass of spermatia. At the protruding apex the spermogonium is provided with a pore through which the spermatia emerge. Spermogonia are irregularly globose and measure $42-67 \mu$ (av. 52.7μ) in diameter and $54-67 \mu$ (av. 59.2μ) in height.

Within the pseudoparenchyma filling the perithecial initial two or three enlarged ascogonial cells are formed. They are not noticeably more deeply staining than the surrounding cells but are conspicuous because of the single, extremely large nucleus in each cell. Trichogynes have been found projecting from the apex of the perithecial initial, but their connection with the ascogonia has not been traced. During the winter the perithecia continue to enlarge, and in the following spring a fascicle of asci pushes up from the base of the perithecium into the central pseudoparenchyma which is crushed and disintegrated. At maturity the perithecium is spherical with a short, ostiolate neck at the apex which protrudes through the epidermis. Perithecia measure $61-93 \mu$ (av. 71.2μ) in diameter and $70-115 \mu$ (av. 86.7μ) in height. The wall is composed of one to three layers of brown, thick-walled cells. The central cavity is occupied by a cluster of clavate, aparaphysate asci, each containing eight irregularly biseriate ascospores. The asci measure $44.8-60.8 \times 9.3-9.9 \mu$. The ascospores (Fig. 3, B) are hyaline to pale green, 1-septate, slightly constricted at the septum, fusoid or clavate, straight or slightly curved, rounded at either end, and measure $14.3-24.2 \times 2.7-4.5 \mu$ (av. $19.7 \times 4.2 \mu$). Each cell of the ascospore usually contains a large globule near the septum and one or several smaller ones near the tip. When the perithecia are moistened, the ascospores are forcibly discharged. They germinate readily, sending out a germ tube from each end of the spore.

In 1947 mature ascospores were not found in field material until May. This was an unusually late season, however, and doubtless they usually develop earlier. When leaves were brought in from the field and kept in a moist chamber, ascospores developed in April.

The fungus probably is carried to new localities on planting stock. Although the crowns are dug and transplanted in late winter or early spring after the leaves have fallen, it is possible that fragments of old diseased leaves containing the sclerotia-like fruiting bodies of the fungus are transported with the young plants.

CONTROL

No studies on the control of angular leaf spot of kudzu have been made but a knowledge of methods of propagating this plant suggests certain precautions that might be helpful.

During the 1930 decade considerable seed was imported from Japan. Because seed has not been available from this source in recent years, propagation by means of crowns has become the accepted practice. It seems probable that seed may soon become more abundant and again come into common use. Kudzu seeds need scarification before planting and the method reported by Tabor (6), namely, soaking the seed in concentrated commerical sulfuric acid for one hour at 60° F., should serve not only to scarify the seed coats but to disinfect them as well. It is not known whether the fungus ever enters the seed, but this seems improbable. So far as known, it attacks only the leaf tissue.

The difficulty of completely removing the fungus from other propagating material is obvious. Johnson (4) recommends soaking the vine cuttings for 20 minutes to one hour or longer in a 0.1 per cent solution of potassium permanganate just before planting to stimulate root growth. It seems certain that this chemical at the recommended strength would have little if any fungicidal value. It is possible, of course, that other fungicides could be found that would be effective and not injure the tissue. Perhaps for the present it would be sufficient to use every precaution to free the propagating material of all old leaf tissue and soil particles that might harbor the fungus.

If, as now seems probable, this disease has been in the United States for a number of years without having attracted more attention, control measures, other than the precautionary ones suggested above, may not be needed. Yet in some instances observed the plants had lost a considerable proportion of their foliage. The fact that kudzu is a perennial and the planting remains in the same location for several years should offer excellent opportunity for the fungus to become more abundant and more destructive each year. On the other hand, the plant grows so rapidly that a severe epiphytotic would be required to destroy it.

DISCUSSION

Since this disease has been reported from Formosa (8) and China (7), it seems probable that it is widespread in the Orient. No specific reference to the occurrence of angular leaf spot in Japan has been found. All of the kudzu seed used in the United States from 1910 to 1940, or until the supply was cut off, is said to have come from Japan. It is not known to the writers whether some of this seed originated from Formosa, which has been classed by some Atlases as south Japan. At any rate the most logical explanation of the presence of this disease in the United States seems to be that it was imported with the seed. This suggests that the fungus is probably widespread in this country, especially in sections where the imported seed was planted. The recent report of its occurrence in Mississippi and the present report of its presence in Georgia and Alabama support such a supposition. It seems probable that this disease has been over-looked previously because of its similarity in appearance to certain stages of halo

spot. The kudzu plant grows so rapidly that the older leaves are more or less covered. The angular leaf spot develops rather slowly so that often it probably is not sufficiently abundant to attract attention until fairly late in the season.

SUMMARY

The symptomatology and etiology of a leaf spot of kudzu (*Pueraria thunbergiana* Benth.) herein designated as angular leaf spot, is described. The causal fungus appears to be identical with *Cercospora puraricola* Yamamoto. Until recently the disease had been reported only from the Orient. A recent report by Bain and Presley, together with the present one, show that the disease is present in Mississippi, Georgia, and Alabama, and probably is much more widespread than was formerly supposed. Lesions, which are confined to the leaves, are 1 to 10 mm. in diameter, dark brown, often with a yellowish margin, and, during damp weather, with numerous conidial tufts largely confined to the upper surface. Lesions may coalesce and involve large areas of the leaf and cause defoliation.

The perfect stage of the causal fungus was found on overwintered kudzu leaves. The ascospore stage was isolated, and its appearance in culture and its pathogenicity prove that it is identical with the conidial stage associated with the disease on living leaves. The characteristics of the perithecial stage place the fungus in the genus *Mycosphaerella*, and it is described as new under the name *M. puraricola*.

U. S. DEPARTMENT OF AGRICULTURE
       AND
GEORGIA AGRICULTURAL EXPERIMENT STATION

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CANKER OF TUNG TREES CAUSED BY PHYSALOSPORA RHODINA

JOHN R. LARGE[1]

In the late summer of 1944 a canker disease of tung nursery trees caused by *Diplodia theobromae* (Pat.) Nowell[2] (*D. natalensis* Evans) was found on about 300 trees in a tung nursery in Pearl River County, Mississippi. Later the same symptoms were observed in an extensive 2-year-old orchard in the same county. At four different points in this orchard one or two trees were severely affected and several had cankers on one or more branches. In October the disease was observed on several 8-year-old trees in another large Pearl River County orchard. Subsequently about 75 trees, affected in various degrees, were found in a newly planted orchard in St. Tammany Parish, Louisiana. During 1945 and 1946, infected trees have been found in four additional orchards in Pearl River County.

LITERATURE

Eddins and Voorhees (1) inoculated tung and 30 other host plants with mycelia from pure cultures obtained from pycnospores of the imperfect stages of *Physalospora zeicola, P. rhodina,* and *Diplodia tubericola*. Edson and Wood (2) reported *Diplodia natalensis* as a pathogen causing crown girdle of tung. Verrall (9), after inoculating small tung trees with 10 isolates, concluded that none of the isolates used was sufficiently pathogenic to cause more than small temporary cankers that soon callused over. Stevens and Wilcox (8) found that the pycnospores of many species of *Diplodia* from various tropical hosts in different parts of the world, were identical, and the cultures showed the unusual characteristics of growing at 37° C. and turning potato-dextrose agar pink at that temperature. Pole-Evans (4) called attention to the fact that *Diplodia natalensis* (*D. theobromae*) fruits more readily on apples than on citrus fruit. Ramsey *et al.* (5) inoculated sweet potato, apple, and orange with the onion *Diplodia*, and gave detailed description of the development of the disease on the apple.

SYMPTOMS

During late summer and early fall the disease as observed on tung in Mississippi and Louisiana was characterized by black sunken cankers on the trunks, limbs, twigs, shoots, or leaf petioles. Sometimes the canker girdled the trunk, killing the tree back almost to the soil level, after which

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[2] Specific determination of the organism was made by John A. Stevenson, Division of Mycology and Disease Survey, U. S. Department of Agriculture.

new shoots developed just below the canker. In other cases only a single branch, such as the tip of the central leader, was affected. When a canker girdled the trunk or branch, the leaves wilted suddenly and the affected part died. The following spring many of the dead branches developed ash grey cankers with numerous black perithecia scattered over the surface. This is characteristic of *Physalospora rhodina,* as described by Voorhees (10).

In the fall dead branches were collected for study and pycnidia containing single-celled hyaline spores 13–27 $\mu \times$ 6–15 μ, averaging 24 $\mu \times$ 14 μ and resembling spores of *Macrophoma,* were consistently observed on these branches. This material was studied a year later and several dark bicellular spores 19–24 $\mu \times$ 10–12 μ, averaging 22 $\mu \times$ 11 μ, were observed. Fawcett (3) stated that this change from single celled spores to bicellular spores is typical of the fungus *Diplodia natalensis* (*D. theobromae*).

Cultures from spores obtained from tung material, as well as tissue cultures from the margin of the canker and from the black pith of the diseased branch, developed dark mycelium which gradually turned the culture brown to dark brown, appearing black in mass, which is characteristic of cultures of the fungus *Diplodia natalensis* (*D. theobromae*). Cultures from these isolations were used for pathogenicity studies.

Perithecia which contained hyaline asci and ascospores characteristic of *Physalospora,* were observed on the ash grey twig cankers. The asci were 63–75 $\mu \times$ 20–22 μ; the ascospores 15–24 $\mu \times$ 8–9 μ. When sectioned transversely, the contents of the mature perithecia were almost snow white. Shear, Stevens, and Wilcox (6) consider this a characteristic which makes it possible to identify the genus *Physalospora* in the field.

PATHOGENICITY

Cankers of the disease have been produced on stems of one-year-old tung trees inoculated in the greenhouse with isolates of the fungus growing on agar medium. The pathogen was later reisolated from the inoculated stems. In all cases 10-day-old cultures of the fungus on potato-dextrose agar, in Petri plates, were employed for the inoculations. A block of the agar culture approximately a half inch square was applied to the surface of the tung stem, which had been injured by punctures with a sterile needle. The agar medium was then covered with wet cotton, tied around the stem with string and the entire stem tip inserted into a paper bag which was tied shut below the point of inoculation. The bags and cotton were removed three days after inoculation . Controls were treated similarly to the above, but sterile agar blocks were used. The results of the inoculations are shown in table 1.

The portion above the canker of the two trees first infected died within 3 days after inoculation and manifested the characteristic darkening of the

pith and ashy grey stem areas covered with black pycnidia which appeared snow white in cross section. The trees that became infected later developed symptoms of the disease very slowly. After three months the cankers formed on the inoculated stems were only 2 cm. long by 1 cm. wide. In no case did the canker girdle the stem. Evidently the difference in rate of development of the disease was due to the vigor of the inoculated plant. Voorhees (10) and Verrall (9) noted the slow extension of the infection on inoculated woody stems, due to the formation of callus tissue which prevented further spread of the fungus.

Reisolations of the fungus, three months after inoculation, from 10 cankers gave typical growth of *Diplodia* on potato-dextrose agar, identical with the parent cultures.

The injuries on the check trees healed within three weeks.

TABLE 1.—*Results of inoculating young tung trees with pure mycelial cultures of Diplodia theobromae*

Method of inoculation	Number of trees inoculated	Number of trees infected after		
		15 days	90 days	350 days
Mycelial cultures placed over wounds in the stem	60	2	28	36
Control: stem wounded and covered with sterile agar	10	0	0	0

EFFECT ON APPLE FRUITS

Ripe apples inoculated with the tung *Diplodia* developed typical black rot symptoms, which Ramsey *et al.* (5) have described as follows: "Apples inoculated through a side wound developed an extensive brownish-black lesion within a week, and ultimately the whole fruit was converted into a black, moderately firm mummy. Pycnidia were formed in the peel and the internal tissues were full of large hyphae, typical for *Diplodia*." Single-celled hyaline spores were observed in the pycnidia, one week after inoculation, and dark colored bicellular spores were observed a month later. These results are in agreement with those of others (1, 5, 7, 9, 10) who have shown that strains of *Diplodia* from a wide variety of crop plants are similar morphologically and produce similar symptoms when cross-inoculated on a number of different host plants.

EFFECT OF TEMPERATURE ON CHROMOGENESIS

Stevens (7) found that cultures of *Diplodia* showed the unusual characteristic of growing at 37° C., and that many strains turned potato-dextrose agar pink at that temperature. Voorhees (10) observed that stock cultures of several races of *Physalospora rhodina* growing on potato-dextrose agar at approximately 17° C. showed chromogenesis. However, when subcul-

cultures from the stock cultures showing this character were made on potato-dextrose agar in Petri dishes and incubated at 35° C. or below, no color developed.

The cultures of *Diplodia* reisolated from inoculated tung trees showed chromogenesis when grown on cornmeal agar stored in a refrigerator at approximately 17° C., on Difco potato-dextrose agar in test tubes held at 37° C., and on potato-dextrose agar, made according to the formula of Stevens and Wilcox (8), held at 37° C. Cultures growing on rice medium at 37° C. and also at room temperature from 19 to 27° C., showed definite chromogenesis. The tung *Diplodia* grew more rapidly when incubated at 37° C. than at room temperature (table 2).

TABLE 2.—*Growth of tung Diplodia on potato-dextrose agar at different temperatures*

Temperature, degrees C.	Cultures	Average diameter of culture after		
		24 hours	48 hours	72 hours
	Number	*Mm.*	*Mm.*	*Mm.*
17	5	0	0	0
19-27	5	17	34	41
37	5	25	50	76

No spores were formed by the organism when grown on cornmeal agar, potato-dextrose agar, rice medium, sterilized tung stems, and sterilized tung leaves.

On the basis of the present studies, the writer considers the causal organism of the tung disease to be a strain of *Physalospora rhodina*, Cke., the perfect stage of *Diplodia theobromae* (Pat.) Nowell (*D. natalensis* Evans).

SUMMARY

Physalospora rhodina ex Cke., caused a canker disease of tung trees in several nurseries and orchards in Mississippi and Louisiana. The perithecia of this fungus were scattered singly in the bark of the host. When cut transversely, especially before the spores were fully mature, the contents of the perithecia had the white appearance characteristic of the genera *Botryosphaeria* and *Physalospora*. Pathogenicity was demonstrated by inoculation of tung seedlings, which developed both the *Physalospora* and *Diplodia* stages of the organism; cultures were reisolated from the *Diplodia theobromae* stage of the organism. In culture the tung *Diplodia* grew rapidly at 37° C. Cultures growing on rice medium at 37° C. and at room temperature showed definite chromogenesis. No spores were found on any of 5 culture media used. The tung *Diplodia* caused decay of ripe apples. Typi-

cal bicellular *Diplodia* spores were reisolated from the pycnidia, which developed in the peel of the inoculated mummified apple.

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A LEAF–CURL DISEASE OF TOMATO

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A serious disease of tomato characterized by pronounced dwarfing of the leaves has been observed at Delhi and in other tomato growing areas for the last several years. The disease was particularly severe during the winter of 1946–47 and over 90 per cent of the plants in the early sown experimental plots of the Mycology Division of this Institute were found to be infected. In later sowings, however, the incidence of the disease was much lower. The plants, if infected while young, yield almost no crop. The disease occurs year after year and during 1943–44 and 1944–45 over sixty varieties of tomato which were under trial for their resistance to virus diseases were found to be more or less damaged by the disease. Considering its economic importance the study of the disease was undertaken.

SYMPTOMATOLOGY

Infected tomato plants of the variety Sutton's Early Market exhibit clearing of the veins, stunting and marked reduction in leaf size. At the same time the plant has typical mosaic symptoms which vary from mild to severe. The reduction in size of the leaves is more pronounced in the successive leaves and is accompanied by shortening of the internodes with the result that the leaves are crowded together. The leaflets are deformed and their margins may curl inwards or outwards. In a few cases the young leaflets show a tendency towards a corkscrew distortion with the tip forming a type of hook. The younger leaves are pale in color with light green and dark green areas. Sometimes the area between the veins may be completely yellow. Puckering of the leaflets is a common symptom of the disease and is very severe in some plants. The plant has a greater tendency to produce stunted lateral branches which impart a bushy appearance. The plants infected while young remain stunted and seldom attain a size of more than 10 to 15 inches. The disease induces partial or complete sterility of the infected plant, depending on the stage at which infection has taken place. In the cases of late infections the plant bears a few fruits but in earlier infections the symptoms are much more pronounced and the few flowers that are formed bear reduced floral structures which never attain normal size. They seldom open and soon drop off. The flowers that develop after infection are invariably sterile. The diseased plants usually develop purple patches especially on the older leaves, and these vary in intensity in different plants.

MATERIAL AND METHOD

Throughout the work, the diseased material was obtained from infected tomato plants of the variety Sutton's Early Market from the experimental

plots of the Mycological Division.   The same variety of tomato was used throughout the investigation.   The tomatoes, as well as the differential plants, were raised inside an insect-proof house and young, actively growing plants were used for the experiments.   All the apparatus used was carefully sterilized as required for the individual experiment.   For inoculation purposes, carborundum powder was used as an abrasive and grafting was carried out by the wedge method as well as by inarching.

<center>TRANSMISSION OF THE DISEASE</center>

All attempts to transmit the disease with diseased plant extracts to healthy tomato plants were unsuccessful.   More than 50 attempts were made at different times but the inoculated plants failed to develop any symptoms of the disease.   The disease was, however, successfully transmitted by grafting diseased scions to healthy stocks of tomato plants.   Both the wedge-graft method and inarching successfully transmitted the disease within three weeks (Fig. 1, A).   New shoots developed about seven to ten days after the graft.   The healthy stocks first exhibited symptoms of mosaic which were accompanied by a stunting of the plant identical with that of naturally infected plants. '

FIG. 1.   Tomato plants in which the leaf-curl disease has been transmitted by grafting (A) and by white flies (B).

Transmission by insects.   In order to determine the vectors responsible for transmission of the disease a careful survey of the insects present in the field at the time of the appearance of the disease was undertaken and it was found that two types of insects—namely, the capsid, *Engytatus tenuis* Reut.,

and the white fly, *Bemisia gossypiperda* M. Th.—were predominant. It was also observed that during October and November the disease was spreading rapidly in the fields and that the incidence of the two types of insects was very high. It was, therefore, considered necessary to determine whether either of these insects was responsible for the transmission and rapid spread of the disease. The insects used for transmission work were first fed on diseased plants for 24 hours with the help of microcages similar to those described by Storey (1928) in connection with his transmission studies of maize streak virus. After the insects fed on diseased plants they were released on healthy tomato plants inside glass lamp chimneys. The top of a chimney had been covered by muslin. The insects were allowed to feed on healthy tomato plants for two and three days, after which the surviving insects were killed by spraying. The transmission experiments were re-

FIG. 2. Infected White Burley tobacco plants showing mild type of symptoms (A) and pronounced leaf-curl symptoms (B).

peated several times with five to eight insects on each plant. The plants on which capsids had been released never developed any symptoms of the disease. In tests conducted at different times 30 to 100 per cent of the plants on which white flies had been released developed typical symptoms of the disease within 15 to 25 days, depending on the temperature prevalent during the experimental period. During the winter months 25 days were required for the manifestation of symptoms, whereas in March the symptoms appeared in 15 days. Figure 1, B shows a tomato plant infected by viruliferous white flies.

REACTIONS OF DIFFERENTIAL HOSTS

The disease was successfully transmitted by grafting to *Nicotiana tabacum* L. varieties White Burley, German Samsun, and Harrison's Special, *Solanum tuberosum* L. variety Craig's Defiance, *Datura stramonium* L.,

Nicotiana sylvestris Spegaz. and Comes, and *Nicotiana glutinosa* L.   The reactions described are those observed during January to April at Delhi in the insect-proof house in the presence of adequate light and humidity.

A considerable variation was observed in the symptoms produced on *Nicotiana tabacum* var. White Burley.   All the plants invariably showed a type of yellow mottling, but the subsequent symptoms differed in the intensity of stunting as well as in the reduction in size and curling of the leaves in different plants.   The leaf tips in all cases point downwards.   Symptoms may vary considerably in type.   In one type the stunting of the plant is not pronounced but the leaf margins tend to curl downwards.   In all other types stunting of the plant as well as shortening of the internodes and reduction of the leaf size are present in varying degrees.   Vein-banding, rolling downwards of the leaf margins, and thickening of the veins also are common features.   In some plants the leaf surface appears wavy (Fig. 2, A), but in many plants puckering is present.   This may be mild and confined only to

FIG. 3.   A. *Datura stramonium* grafted with diseased tomato shoot.   B. Shoot of an infected potato plant.

portions of the leaf, or may be general and highly pronounced.   Clearing of the veins, when present, is only transient.   In some plants the leaves are thick and brittle and enations in the form of small dark-green thickenings of the veins are present (Fig. 2, B).

The experimental data presented indicate that there may be a number of strains of tobacco leaf-curl virus responsible for the development of the leaf-curl disease in tomato.   If this is true, it appears that the mild as well as the severe forms produce almost similar symptoms in tomato.   A large number of grafting experiments indicate that the virulence of certain strains of the virus diminishes during passage through tomato, and when brought

back to tobacco the strains produce mild reactions.   For instance, the strain that causes the enation type of leaf curl, when transmitted to a White Burley tobacco plant through tomato, produced symptoms resembling the mild type. The enations which are a characteristic feature of the type were absent.

In the German Samsun variety of tobacco the symptoms such as stunting of the plant, shortening of the internodes, reduction in the size of the leaf, as well as yellow mottling, are very pronounced, but the curling of the leaf margins and puckering are absent.   In the variety Harrison's Special the symptoms are more or less similar to the above but the mottling is not pronounced and there is a tendency for the leaf margins to curl downwards. *Nicotiana sylvestris* reacts only mildly to the disease, yellow mottling and vein-banding being the prominent symptoms.   In *Nicotiana glutinosa* L. there is a general yellow mottling, dwarfing, and cupping of the leaves.

Datura stramonium L. reacts violently to the disease: stunting is accompanied by a general yellow mottling, dwarfing, curling and corkscrew distortion of the leaves (Fig. 3, A).

In *Solanum tuberosum* L. (var. Craig's Defiance) stunting of the plant is accompanied by mottling, dwarfing, and thickening of the leaves, which are more or less crowded together and appear crumpled (Fig. 3, B).

DISCUSSION

The symptoms on differential hosts, particularly those on tobacco, indicate that this disease of tomato is caused by the tobacco leaf-curl virus since the curling of the leaves and development of enations are very typical of this disease on tobacco.   This view is further confirmed by the fact that the disease is transmitted by *Bemisia gossypiperda*, vector of the tobacco leaf-curl virus.   This virus has been experimentally transmitted by a number of workers, *e.g.*, Thung (9), Storey (8), Pal and Tandon (4), and McClean (3), but natural occurrence of the disease on tomato has been mentioned only by Pruthi and Samuel (5).   The symptoms of the disease as it occurs on tomato have not, however, been fully described by the authors.   Bertus (2) records the occurrence of a leaf curl and stunting disease of tomato which is transmitted by white flies and grafting but not through sap.   It is likely that the disease that Bertus described is caused by the tobacco leaf-curl virus.   The natural occurrence of the disease in wild varieties of tomato has been observed in Nyasaland (1).

As early as 1932 Thung (9) described three types of leaf curl on tobacco. The presence of more than one strain was also observed by Storey (7), when he found that there was considerable variation in the severity of the symptoms produced.   In his transmission experiments his tobacco plants generally developed the disease only mildly.   He suspected that the variations in the individual reactions of the plant may play a part and also that there may be a number of strains of the virus of varying virulence.   Pal and Tandon (4) in their studies on tobacco leaf curl have differentiated five different types of leaf-curl virus, but from our observations it appears that a

much wider variation exists.   McClean (3) states that in the course of the transmission of leaf curl of tobacco, a new type of the disease arose that produced milder symptoms on tobacco and other hosts.   He distinguished severe, mild, and latent forms and stated that the latter two had their origin in plants infected with severe leaf curl.   He also established the fact that in the process of transmission of virus from a plant with severe leaf curl plants may acquire weaker forms of virus which produce a milder form of disease.

The suggestion of Storey that the variations in the individual reaction of the plant may play a part in the variation of the severity of the disease also must be considered.   Moreover, there is a wide variation in the symptoms produced in different varieties.   In our experiments it was observed that the tobacco plants of variety White Burley when grafted to other varieties of tobacco plants already infected with leaf-curl virus developed different symptoms.

SUMMARY

A virus disease of the tomato which causes dwarfing, puckering of the leaves, vein-clearing, excessive branching, and stunting of the plant has been studied.

The disease can be transmitted by grafting and by *Bemisia gossypiperda* but not by inoculation with juice of infected plants.

The causal virus has been identified as that of tobacco leaf curl and inoculations to tobacco indicate that a number of strains of this virus may be involved but that all produce almost identical symptoms on tomato.

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INOCULATION EXPERIMENTS WITH POLYPORUS SCHWEINITZII[1]

SIGMUND BERK [2, 3]

Polyporus schweinitzii Fries is considered primarily as a wood destroying organism that causes a root and butt rot in conifers. Although the literature gives a wide range of coniferous hosts for the fungus (7), there are only two reports of its occurrence on hardwoods. Rhoads (6) found it on a charred stump of *Eucalyptus globulus* and Hubert (4) reported it on red gum and oak.

This fungus has been found by Dr. H. H. York to be very destructive on forest plantings of white and red pine on the watershed of Hemlock and Canadice Lakes, N. Y. The land, originally forested with mixed hardwoods and conifers (chiefly white pine and hemlock), was cleared about 75 years ago for cultivation of beans, potatoes, wheat, oats, and timothy. The coniferous plantations were established about 10 to 25 years ago on old abandoned fields. *Polyporus schweinitzii* has been active in some of these forest plantings for at least 15 years. The fact that this fungus is so widespread suggests the following questions: Have the infections occurred from outside sources of spores of the fungus, or has this organism persisted saprophytically in the soil or as a weak parasite on the roots of hardwoods and field plants? The writer undertook a series of experiments to determine whether or not the fungus can parasitize the roots of various seedling hardwoods and herbaceous plants.

INOCULATION OF SEEDLINGS GROWN IN POTS

Series A, Hardwood Seedlings. Soil used in Series A was obtained from the upper 8 inches of a forest planting of white pine near Springwater, N. Y., where *Polyporus schweinitzii* is widely epidemic. This soil had a pH of 6.0 and a colloidal content of 21 to 50 per cent. The soil was disinfected by wetting with a solution of formalin (1 oz. to a quart of water). The disinfectant was permitted to vaporize from the soil by occassional raking and exposure to air and sun for 3 days.

The inoculum used in Series A to D inclusive was prepared by filling 10-cm. Petri plates two-thirds full of finely divided pine needle duff from forest plantings of white pine near Springwater, N. Y. This duff, saturated with a 2 per cent solution of Fleischmann's Diamalt, was autoclaved for

30 minutes at 15 lb. steam pressure and inoculated at the center of the dish. After 2-4 weeks incubation at room temperature in diffused light, the fungus grew well through the duff, and the inoculum was ready for use. Unless stated otherwise, variant No. 12 of *Polyporus schweinitzii* was used in this study.

Four 6-inch clay pots comprised a series, two pots with treated soil and two with untreated soil. One pot with untreated and one with treated soil were inoculated just prior to planting by the addition of needle-duff inoculum from one Petri plate. Equivalent amounts of the noninoculated needle-duff were added to one pot with untreated and one with treated soil. Pots of soil that were inoculated with the fungus prior to planting received a second similar inoculation in the soil, 25 weeks after the hardwood seeds were planted.

The following seeds, which were not surface-sterilized, were sown on the surface of each pot and covered with soil: American elm (*Ulmus americana L.*), Scotch elm (*Ulmus glabra Huds.*), and silver maple (*Acer saccharinum L.*). All seeds and seedlings in Series A to F inclusive were grown in the greenhouse.

A striking difference in growth was observed in the hardwood seedlings after 11 weeks (Fig. 1). Dwarfing occurred in the plants grown in formal-

FIG. 1. Seedlings of American elm in (A) nontreated, noninoculated forest soil, (B) formaldehyde treated soil inoculated with *Polyporus schweinitzii*, (C) nontreated soil inoculated with *P. schweinitzii*, and (D) formaldehyde treated soil without fungus inoculum.

dehyde treated soil — in length of shoot and root systems, and in number and size of leaves per plant.

Isolations were made from the roots after 31 weeks when there were no open lesions of decay but portions of lateral roots had been killed. The infected roots were surface-sterilized by the following methods: a 2-minute

immersion in an aqueous solution of 0.1 per cent mercuric chloride, followed by at least 3 rinses in sterile distilled water; 5 to 20 seconds immersion in Superoxol (30 per cent hydrogen peroxide) ; and a 2-minute dip in 3 per cent hydrogen peroxide. The surface-sterilized roots were plated in tubes of 2 per cent Fleischmann's malt agar medium. A 2-minute application of 0.1 per cent aqueous solution of mercuric chloride with 3 rinses in sterile distilled water was found to be the most reliable root surface sterilization method. Discolored, swollen, dwarfed, and killed roots did not yield a single positive culture of *Polyporus schweinitzii*, but did yield species of *Fusarium, Trichoderma, Alternaria, Macrosporium,* and other fungi.

Series B. Lupine and American Elm Seedlings. Six-inch clay pots were filled with 1 part soil from Springwater, N. Y., 5 parts leaf humus, and 1 part sand. Inoculum from a 10-cm. Petri dish two-thirds full of pine needle duff saturated with 2 per cent Fleischmann's malt was added to each pot. The pH of this soil mixture after autoclaving was 6.4.

American elm and lupine (*Lupinus albus* L.) seeds were surface-sterilized by immersion for 12 to 45 minutes in an aqueous solution of calcium hypochlorite, somewhat similar to the method used by Wilson (8). The excess disinfectant was not washed from the seeds, since it does not interfere with germination. The pine needle duff in the pots was inoculated with *Polyporus schweinitzii* when the surface-sterilized seeds were sown.

Root isolations from lupine were made 55 days after sowing and from American elm 75 to 157 days after sowing. The lupine seedlings had slight lesions on the root crown and the tip of the tap root was killed. A few of the elm seedlings had lesions on the primary roots. *Polyporus schweinitzii* was not isolated from the diseased roots.

Series C, American Elm inoculated with Variants of Polyporus schweinitzii. Four-inch clay pots were filled with potting soil and a mixture of 2 parts potting soil to 1 part finely ground pine needle duff. The inoculum, prepared in Petri dishes as described previously and consisting of variants No. 10, 12, and 40E of *Polyporus schweinitzii* alone and in various combinations, was added to each pot at the time of sowing the surface-sterilized American elm seeds. Variants No. 10 and 12 were obtained by Childs (2) from infected *Pinus strobus* L., Springwater, N. Y., and No. 40E from *Pinus mughus,* Central Experimental Farm, Ottawa, Ontario. Variants of *P. schweinitzii* were used in this series, since it was thought that variant No. 12 used in series A and B might have been weakened by long and continued growth on artificial media or that possibly it was not a virulent "race" of the parasite. Isolations made from diseased roots after 12 weeks growth were negative for *P. schweinitzii.*

Series D, Liriodendron tulipifera Seedlings. Three-inch seedlings of *Liriodendron tulipifera* were transplanted into six-inch pots containing potting soil. The roots of each plant were surrounded by pine needle inoculum of variants No. 12 and 40E alone and in combination. Isolations

made from rotted primary roots after 10 weeks growth were negative for *Polyporus schweinitzii* and yielded primarily species of *Fusarium*.

Growth Rate of Fungi Isolated from Roots. The growth rates on 2 per cent Fleischmann's malt agar of a species of *Fusarium* and one of *Penicillium* isolated from infected roots were compared with that of variant No. 12 of *Polyporus schweinitzii*. The species of *Fusarium* grew twice as fast and the species of *Penicillium* three times as rapidly as *P. schweinitzii* on tue malt agar medium. These results indicate the difficulty of isolating *P. schweinitzii* when rapidly growing soil organisms are present.

INOCULATION OF SEEDLINGS GROWN UNDER ASEPTIC CONDITIONS

Series E, Lupine and Bush Beans Grown in Pine Needle Duff. Test tubes (38 × 200 mm.) were one-quarter filled with finely ground pine needle duff saturated with 2 per cent Fleischmann's malt and autoclaved 30 min. at 15 lb. steam pressure. Surface-sterilized seeds of lupine (*Lupinus albus* L.) and bush bean (*Phaseolus vulgaris* var. *humilis* Alef.) were transferred to the pine needle duff in the test tubes. The duff was inoculated with variant No. 12 of the fungus, before, after, and at the same time as sowing the seeds.

The lupine control plants survived 40 days while the bush beans lasted 47 days under the growing conditions of this series. Autoclaved pine needle duff was somewhat toxic to the seedlings as evidenced by the reaction of the roots to the medium; *i.e.*, the appearance of adventitious roots above the surface of the duff. Examination of lupine roots 21 to 40 days after sowing and bush beans 28 to 43 days after sowing showed that the root crown and lower part of the stem had lesions, while the primary and secondary roots were necrotic. Severest infection occurred where the fungus mat was in direct contact with the root system. Although shoot development was good, the root system was no longer than 3 or 4 cm. and consisted of a thick primary root with few laterals. Diseased roots were grayish-black or dark brown and almost completely rotted by the fungus (Fig. 2). Isolations were made by dipping diseased portions of roots in 95 per cent ethyl alcohol and plating on malt agar. *Polyporus schweinitzii* was isolated from the roots of lupine and bush beans.

Root material was preserved in dilute formalin and fixed in a saturated aqueous solution of salicylic acid plus chromic sulfate and formaldehyde (3). A histological study of roots from this series revealed the presence of fungus hyphae in the cortical cells of lupine (Fig. 2). However, it is not known whether the fungus penetrated the roots while they were alive or after their death.

Series F, Lupine Seedlings Grown in Soil. Surface-sterilized lupine seeds were grown under aseptic conditions in test tubes (38 × 200 mm.) containing a mixture of 6 parts of potting soil and 1 part of pine needle duff saturated with distilled water and autoclaved one hour at 15 lb. steam

pressure. The cultures were inoculated with variant No. 12 of the fungus
at the time of sowing the seeds.

FIG. 2. Necrotic roots of lupine caused by *Polyporus schweinitzii*. Above: A
from Series F grown in autoclaved soil; B from Series E grown in pine needle duff
saturated with 2 per cent Fleischmann's malt. Below: Photomicrograph showing hyphae
of *P. schweinitzii* in cortical cells of *Lupinus albus* L. (Mag. 1220×).

After 3 weeks' growth, discolored areas were present on the root crown and primary root. Laterals near the surface of the soil and in contact with the pine needle inoculum were killed (Fig. 2). *Polyporus schweinitzii* was isolated from such dead laterals. The fungus was not isolated from the primary root or from secondary roots more than 2 cm. below the surface of the soil.

GROWTH OF P. SCHWEINITZII IN STERILIZED SOILS IN THE LABORATORY

Boyce (1) questioned the ability of the fungus to grow in natural soil. To study the growth or survival of the fungus in soils, horizontal glass tubes (25 × 320 mm.) were utilized. The following types of soil mixtures saturated with distilled water were added to the tubes: Soil from Springwater, N. Y. (Series A); 4 parts soil from Springwater, N. Y., plus 1 part finely ground pine needle duff; and potting soil from Series C and D. After autoclaving for one hr. at 15 lb. steam pressure, the soils in the tubes were inoculated at one end and incubated in the laboratory at 22° C. in a horizontal position in total darkness.

Table 1 shows the rate of growth of *Polyporus schweinitzii* in the three

TABLE 1.—*Rate of growth of Polyporus schweinitzii in soils in glass tubes*

Type of soil	pH	Days after inoculation			
		26	36	43	53
		Growth in mm.			
From Springwater, N. Y.	5.8	120	170	190	320 a
4 parts soil from Springwater, N. Y., plus 1 part pine needle duff	5.1	170	240	. . ..	320 a
Potting soil	6.1	10	20	20	22

a Mycelium completely filled the 320-mm. tubes.

types of soil mixtures. It grew most rapidly in soil from the Springwater region plus pine needle duff. *P. schweinitzii* was isolated when the mycelium had grown through the soil to the opposite end of the tube.

Attempts to isolate the fungus from inoculated soils (Series A to D) in pots in the greenhouse failed.

DISCUSSION

The results of this investigation indicate that *Polyporus schweinitzii* is not capable of parasitizing roots of the hardwood seedlings under the conditions of this study. Although there were signs of root decay and of dead laterals, isolations from such regions did not yield the fungus.

In autoclaved soils of the various series inoculated with the fungus, the organism is not only capable of surviving but of actually making rapid growth in some. However, in sterilized soils, the organism does not en-

counter normal soil competing fungi which enter when the soil is exposed to the atmosphere. Species of *Fusarium, Penicillium,* and other fungi isolated from the roots grew more rapidly than *Polyporus schweinitzii* in culture. It is possible that the inoculum may have been completely overrun by such fast growing forms and the fungus suppressed before it could penetrate host tissue. Assuming the destruction of primary inoculum and the possibility that *P. schweinitzii* can parasitize only when the host has reached a certain stage of development, the soil in Series A was inoculated a second time. Attempts to isolate the fungus from the roots of the hardwood seedlings growing in the reinoculated soil also failed.

Rayner (5) states that Melin working with mycorrhizal fungi found *Fusarium* frequently outgrowing the true endophyte. It is possible that *Polyporus schweinitzii* penetrating host tissue offered an atrium for a secondary fungus like *Fusarium* which crowded out the primary parasite because of a faster growth rate. Considering the large number of isolations of *Fusarium* obtained, this concept may be worth further testing.

In autoclaved pine needle duff saturated with 2 per cent malt (Series E), conditions of growth apparently were optimum for the fungus but very unfavorable for the host. It seems from the work of Wean (7) that the fungus produces succinic acid which may weaken or kill root tissue and allow the hyphae to penetrate the cells with little difficulty. Wean (7) found that the fungus entered directly through living epidermal and cortical cells and through the base of lateral roots of *Pinus strobus* L. The author also found fungus hyphae in the cortical cells of lupine in Series E.

Results from inoculations in Series F seem to indicate that the fungus was weakly parasitic. The writer favors Hubert's (4) views that the organism may become parasitic only when the host is growing under very unfavorable environmental conditions. Infection of lupine roots was obtained under aseptic conditions in the absence of competing soil fungi. However, it was impossible to isolate the fungus from the roots of lupine plants growing in soil exposed to the atmosphere. It is possible that *Polyporus schweinitzii* may have been overgrown or suppressed by more rapidly growing organisms which are present in soils exposed to the air.

The results of these experiments do not preclude the possibility that the organism may live in agricultural soils for a long time after the land has been cleared, especially when the soil is rich in humus materials.

SUMMARY

Herbaceous and hardwood seedlings grown in various soils were inoculated with *Polyporus schweinitzii* in order to test the ability of the fungus to parasitize living root tissue. The *Polyporus* could not be reisolated from roots, possibly because a species of *Fusarium* and one of *Penicillium* obtained from infected roots have growth rates that are much greater, on 2 per cent Fleischmann's malt agar, than the growth rate of *P. schweinitzii.*

Infection characterized by necrotic lesions was obtained in bush bean and lupine seedlings grown under aseptic conditions in a medium of autoclaved finely ground pine needle duff saturated with 2 per cent malt and inoculated with the fungus. Lupine grown in soil under aseptic conditions showed a slight infection of lateral roots near the surface of the soil.

The fungus can survive and grow rapidly in certain types of sterilized soil. Best growth was obtained in soil from Springwater, N. Y. (pH of 5.8 and a colloidal content of 21–50 per cent). The most rapid growth was in soil from the Springwater region containing a small amount of pine needle duff.

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WHITE ROT OF SHALLOT

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INTRODUCTION

White rot (*Sclerotium cepivorum* Berk.) of shallot, garlic, and onion was first observed in Louisiana in 1942 (21). The only known infestations at present are on a few small farms scattered in that area of the State which extends for about ten miles along the east bank of the Mississippi River about halfway between Baton Rouge and New Orleans. Shallots are grown intensively in this area along with some garlic and onions. Most of the farms are small and rotation is almost impossible. Although the infected areas are rather limited now, white rot is a potential threat to the entire shallot, garlic, and onion growing business in the southern part of the State. These crops are grown in the cooler season of the year when conditions are most favorable for the development of the disease.

This paper includes studies of white rot as it occurs on shallot in Louisiana. The pH relationships of the causal fungus were studied in culture on liquid and solid media. Control measures tested in naturally infected soil under field conditions included (1) the application of lime and other chemicals to the soil and (2) use of certain seed treatment fungicides. A number of shallot varieties were tested for resistance to white rot.

SYMPTOMS ON SHALLOT

Affected plants first show a slight wilting of one or more leaves. The adjoining leaves gradually wilt and the tips turn yellow and die back. The plants may gradually decline for several days or there may be a rapid wilt and collapse. Shallot bunches often remain severely diseased on one side for some time while the other side may be healthy.

The stunted, yellow, wilted, diseased and dead plants are conspicuous among the dark green healthy bunches. Infected plants can be pulled from the soil easily because of the decayed root systems. The roots rot off at the crown, and the basal portions of the scales become softened and slough off. The fungus often causes a semi-watery decay of the scales not unlike that caused by *Sclerotinia sclerotiorum* on other vegetables. The white to grayish mycelium of the fungus develops on the diseased portions of the plant below the soil line, and soon large numbers of small, black sclerotia appear. Typical infected shallots are shown in figure 1. Most of the affected shallot plants die before any bulbs are formed. Occasionally some bulbs are formed if infection occurs late in the season. However, such bulbs, even if slightly infected, almost invariably decay within a few weeks after harvest.

SEASONAL DEVELOPMENT

Walker (30) demonstrated that in general the disease on onion was limited to temperatures below 24°C, and developed best between 10° and 20° C. A similar relationship appears to be true in shallots. The earliest appearance of the disease observed in Louisiana was the first of December, and the period most favorable for infection lasts until about March first. White rot in Louisiana is definitely confined to the cooler seasons.

FIG. 1. Shallot plants infected with white rot. Bunch on right shows white mycelium on surface.

Under certain conditions shallot plants may become infected, collapse, and die within 20 days. Healthy transplants (green shallot plants) were set out in naturally infested soil in December, 1942. Twenty days later several of the plants were dead. Sclerotia had already formed on the basal portions of the dead plants.

DISTRIBUTION AND IMPORTANCE

White rot of onion and some other closely related plants has been known in Europe since the disease was first described by Berkeley (4) in 1841. According to Walker (29), the disease is commonly destructive to onion and leek in the British Isles, Holland, and France, and to garlic

in Spain and Italy. Bremer (7) in 1934 reported that white rot of onion was widespread in Germany under the name "Flour disease." Among the other countries in which white rot has been reported are Argentina (13), Australia (1), Brazil (6), Egypt (15), and South Africa (9).

According to Walker (29), the first authentic record of white rot in the United States was on onion and garlic from Oregon in 1918. This was followed by a report of the disease on onion in Virginia in 1923. The disease was reported from Kentucky in 1925 (25), New Jersey in 1936 (10, p. 190), and California in 1938 (11). White rot was reported on shallot, onion, and garlic from Louisiana in 1942 (21). However, the disease probably had been present there for some years previous to that date. While the disease has been recorded from a number of different locations in the United States, no extensive infested areas have been found. For this reason no large scale losses have been reported from this country. Small infested areas were found in Louisiana in which the shallot and garlic crops were a total loss. The disease is a potentially serious threat to the growing of onion and garlic and shallot in the State. These crops are all grown during the fall, winter, and spring seasons when temperatures are most favorable for white rot development.

THE CAUSAL FUNGUS

The organism causing the disease was first described by Berkeley (4) in 1841, as a sterile fungus and named *Sclerotium cepivorum*. Voglino (26) studied the organism and renamed it *Sphacelia allii* after he found that definite conidiophores and conidia were produced. Later Walker (29) did not confirm Voglino's description. Cotton and Owen (8) noted the production of microspores which they did not succeed in germinating. The writer has also observed the presence of microspores in old tube cultures on Czapek's agar. Several attempts were made to germinate the microspores without success.

Sclerotia germinate readily under proper environmental conditions. The fungus may live in the soil for several years without the host plants being present. Ogilvie and Hickman (17) reported that onions planted in a field that had been free of onions for at least eight years were severely affected with white rot. Nattrass (16) recommended a ten-year rotation to help control the disease.

Host Range

White rot apparently is confined to plants belonging to the genus *Allium*. Most of the cultivated members of this genus are susceptible to the disease. Walker (29) recorded the following species as being affected: onion (*Allium cepa*), Welsh onion (*Allium fistulosum*), leek (*Allium porrum*), garlic (*Allium sativum*), and shallot (*Allium ascalonicum*). Tims (22) reported the occurrence of white rot on wild onion (*Allium*

canadense). This plant grows wild in many shallot fields in southern Louisiana. Naturally infected plants were found in a shallot field where white rot was prevalent on garlic and shallot. Inoculations with *Sclerotium cepivorum* from shallot showed that the wild onion was susceptible to the disease.

Cultural Studies

Cultures of *Sclerotium cepivorum* were easily obtained by plating sclerotia from diseased shallot plants, or by placing fresh diseased material in a moist chamber and transferring to agar slants the mycelium that developed within 24 hours. A number of strains of the fungus were isolated from diseased onion, garlic, and shallot. Most of these strains varied little in growth characters or pathogenicity. The cultures used in the later tests were all obtained from diseased shallot plants grown in the infected areas around Convent, La.

Variations. Strains of the fungus have been reported that varied in cultural characters as well as in pathogenicity. Walker (29) tested several cultures from various sources and found one culture which produced profuse white mycelium that obscured the sclerotia. He also tested a culture from Brittany that was non-pathogenic on onion. Nattrass (15) reported that a culture from Holland grew more vigorously than one from Egypt, producing a denser more floccose type of mycelium and forming microsclerotia up to 16 days later.

Early in our work two strains of the fungus obtained from shallot varied in certain growth characters, such as rate of growth, numbers of sclerotia, and color of mycelium. These cultures (called A and B) were obtained from separate single sclerotia. Culture A (Fig. 2) produced numerous sclerotia on Czapek's agar with a minimum of mycelium; culture B developed a heavy mat of white mycelium with very few sclerotia. The latter culture also grew more slowly than culture A. Transfers of these two cultures were made at approximately monthly intervals for three years with little apparent change in any of these characters, except that culture B developed a number of sectors. Several inoculation tests were made on shallot with the two cultures. Culture A produced typical white rot symptoms, but culture B caused no injury. Shallot plants from a typical test are shown in figure 3.

The sectors that developed in culture B varied from pure white to a dark smoky gray. The sectors also varied considerably in color of mycelium, rate of growth, and production of sclerotia. Most of them produced few or no sclerotia on Czapek's agar, a medium very favorable for sclerotial development in the normal cultures of *Sclerotium cepivorum*. None of the sectors showed any evidence of parasitism on shallot. Four of the sectors, along with two typical cultures, are shown in figure 4.
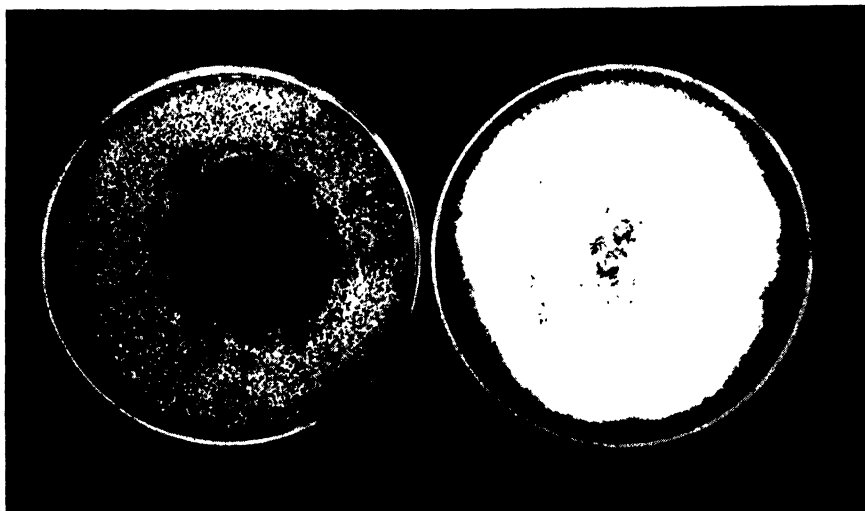
FIG. 2. Cultures of *Sclerotium cepivorum* on Czapek's agar. Culture A on left has typical sclerotial development, while culture B on right has white mycelium with few sclerotia.

FIG. 3. Shallots inoculated with cultures of *Sclerotium cepivorum*. Culture A on left, B on right.

pH relationships. Certain aspects of the disease in Louisiana indicated that white rot might be very restricted in its development in certain soils. It apparently has been present in rather limited areas for a good many years, although no special effort has been made to prevent its spread. Shallots, onions, and garlic are grown regularly in these areas under conditions apparently ideal for the disease to develop and spread from field to field. This failure to spread more extensively seemed to indicate that th causal fungus might be sensitive to comparatively slight differences in soil environment. Most of the white rot infested soils were found to be rather acid (pH 5 to 6.3). Early cultural studies had also indicated that
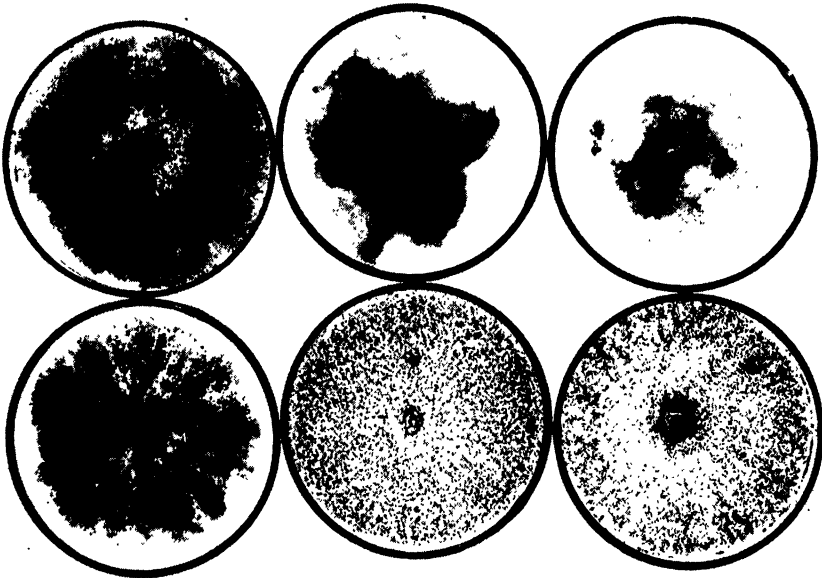
Fig. 4. Cultures of Sclerotium cepivorum. The two plates on lower right show typical sclerotial forms; the others are sectors from culture B.

the fungus was favored by an acid medium. For these reasons a number of tests were conducted in liquid and on solid media to determine the pH relationships of *Sclerotium cepivorum.*

Bacto bean agar was used for a series of pH tests. A pH series ranging from 3.1 to 10.1 was prepared by adding different amounts of N/5 HCl or N/5 KOH to the agar after sterilization. Four plates were poured immediately from each of two flasks at each pH concentration, six of them being inoculated with a suitable culture of the fungus and the other two left as controls. The pH of the agar that remained in the flasks was determined as soon as the plates were poured. The plates were incubated at 22°-23° C. for ten days, after which the agar in all the plates was remelted and pH determinations were made.

The results of a typical test are in table 1. The pH of the nonin-

oculated plates remained relatively stable except in the high alkaline series. But the agar in all the inoculated lots became more acid except in the two most acid series. The fungus growth was good in all plates except those with an initial pH of 9.6 to 10.

Preliminary tests with Richard's solution[1] indicated that the white rot fungus might be definitely limited in growth above pH 7. Several series of tests were made to determine the pH limits for growth of the fungus in this medium. The method of Karrer and Webb (12) was used for determining the amounts of acid or alkali necessary to produce the desired pH range. A series was prepared in which the pH varied from 1.8 to 7.7. The necessary amounts of N/5 HCl or N/20 KOH to bring about the desired concentration were added after sterilization. Six flasks were used at each pH concentration. Four flasks in each lot were inoculated with a

TABLE 1.—*Relation of pH to growth of Sclerotium cepivorum on Bacto bean agar plates*

	Final pH		
Initial pH	Control	Inoculated	Growth of the fungus
3.2	3.1	3.1	Good
3.5	3.5	3.2	Good
4.0	4.1	3.3	Good
5.1	5.2	3.5	Good
7.1	6.7	3.6	Good
8.8	8.4	3.8	Good
9.6	8.9	4.4	Fair
10.1	9.0	6.8	Slight

small disk cut from an agar plate of a rapidly growing culture of *Sclerotium cepivorum*. The other two flasks were kept as controls and the medium was tested for changes in pH at the end of the experiment. The flasks were incubated at 22°-23° C. for 21 days, after which the necessary observations were made and further pH determinations were obtained. The results are in table 2.

Growth of the fungus was definitely limited between pH 2 and 7. There was little change in the pH of the controls, and not much change in the inoculated flasks except at initial pH of 6.2 and 6.3. The fungus grew best and produced sclerotia most abundantly at pH 6.3.

Since the growth of the fungus seemed to be definitely limited below pH 2 and above pH 7 in Richard's solution, further attempts were made

[1] Richard's solution:

KH$_2$PO$_4$	0.5 gm.
KNO$_3$	4.0 gm.
MgSO$_4$ + 7 H$_2$O	0.5 gm.
NH$_4$NO$_3$	10.0 gm.
FeSO$_4$	Trace
Sucrose	30.0 gm.
Distilled H$_2$O to make 1 liter.	

to determine the pH limits more accurately. In a similar test, there was no growth at pH 1.9 and 7.1, traces of growth at pH 2.1 and 6.9, slight growth at pH 6.8, fair growth at pH 2.3, and excellent growth at pH 6.4.

Similar tests were conducted with Czapek's solution[2]. A series of flasks was set up with pH values ranging from 2.1 to 9.8. All of the flasks were inoculated and incubated at 23°-24° C. for 21 days. One set of inoculated flasks was tested after 7 days, another after 16 days, and the r mainder after 21 days. The results in table 3 show that slight changes in pH level occurred after 7 days, but the most important changes took place before the 16th day. The fungus grew well and produced numerous sclerotia at initial pH levels from 5.4 to 7.2. But there was some growth from pH 2.7 to 9.1. Shubbs[3], working in Australia, obtained similar results with *Sclerotium cepivorum* in a beefine-peptone-dextrose medium.

TABLE 2.—*Relation of pH to growth of the white rot fungus in Richard's solution*

| Initial pH | pH after 21 days | | Growth of the fungus |
	Control	Inoculated	
1.8	1.7	1.7	None
1.9	1.8	1.8	None
2.1	2.1	2.1	None
2.3	2.3	2.2	Moderate
6.2	6.2	4.4	Good
6.3	6.4	4.5	Excellent
7.2	6.7	6.7	None
7.5	6.9	6.9	None
7.7	6.8	7.0	None

These limited tests with several media indicated that *Sclerotium cepivorum* grew over a wide pH range on certain media, but was definitely limited on other media such as Richard's solution. Since the white rot fungus would grow on media with such a wide pH range, field control by changing the pH of the soil seemed questionable. However, tests were conducted in which lime was applied to infected soil in an effort to control the disease.

[2] Czapek's solution:

$MgSO_4 + 7 H_2O$	0.5 gm.
KH_2PO_4	1.0 gm.
KCl	0.5 gm.
$FeSO_4$	Trace
$NaNO_3$	2.0 gm.
Sucrose	30.0 gm.
Distilled H_2O to make 1 liter.	

[3] Personal communication.

CONTROL

Apparently no completely satisfactory method has been developed for the control of white rot on any of its hosts. Most of the control experiments have been reported on onion from Great Britain. Very little work has been reported on control of the disease in shallots. Asthana (3) reported one test in which lime was applied to the soil, but the results were inconclusive. Somewhat later Tims (24) mentioned that the addition of lime and other chemicals including Semesan and mercuric chloride to the soil reduced the amount of white rot in shallots.

Numerous onion varietal resistance trials have been reported from the British Isles. In 1920 Cotton and Owen (8) reported that shallot and leek apparently were more resistant than onion. However, all the evidence obtained in Louisiana indicates that the shallot is rather susceptible. Nattrass (16) reported onion varietal tests in infested soil. There was some

TABLE 3.—*Relation of pH to the growth of the white rot fungus in Czapek's solution*

| Initial pH | pH of inoculated flasks after | | | Growth |
	7 days	16 days	21 days	
2.2	2.1	2.1	2.3	None
2.7	2.8	3.6	4.5	Fair, few sclerotia
5.4	3.9	4.4	4.6	Good, numerous sclerotia
6.5	6.5	4.8	4.5	Good, numerous sclerotia
7.2	7.2	5.0	4.8	Good, numerous sclerotia
8.1	7.9	7.2	6.6	Fair, few sclerotia
9.1	8.2	7.4	7.4	Slight
9.5	8.5	8.5	8.4	Trace
9.8	8.7	8.7	8.6	None

variation in percentages of diseased plants but no evidence of a satisfactory degree of resistance. In 1930 Smith (19) reported that "a satisfactory degree of resistance to white rot has been shown by the 'white welch' onion variety in Lancashire and Cheshire." Later reports (2) indicated that onion varieties with marked resistance to white rot have been developed as a result of several years' tests at Manchester University. In 1941 Ogilvie and Walton (18) listed several onion varieties that had moderate resistance to white rot.

The use of chemicals for control of white rot of onion has also been reported. In 1930 Wingard (32) treated onion bulbs with formaldehyde before planting them in infested soil. This treatment gave no control. Ogilvie and Hickman (17), working in England, reduced the white rot in diseased soil by the application of hydroxymecuri-chlorophenol at the rate of

1 oz. per sq. yd. of soil, but they did not obtain satisfactory control. Later Booer (5) reported promising results from England where he applied mercurous chloride dust to the drill with the onion seed. One pound of the 4 per cent dust to 25 yards of row gave 7.9 tons of onions per acre compared to 1.58 tons on untreated rows. Rotation has been recommended for white rot control, but few data have been reported to show the effectiveness of such measures. Shubbs[4] in Australia mentioned "two cases where market g. rdeners have eliminated losses due to white rot disease by the liberal use of lime, rotation and clean cultivation." Asthana (3) found that comparatively large amounts of potassium sulphate reduced the amount of disease in onions; and lime applied at the rate of 2-5 tons per acre also reduced the incidence of white rot.

All of the experiments for the control of white rot of shallot were conducted on a small block of naturally infested soil near Convent, La. The block consisted of 6 rows six feet wide and 350 feet long. The plot was heavily infested with the white rot fungus for about half its length while the other end was more lightly infested. A crop of garlic grown in this block of land in 1942 was almost totally destroyed by the disease. Shallots were planted in this plot in 1943, 1944, and 1945. A portion of the block was used in the lime tests during the three-year period. Parts of the remainder were used for miscellaneous tests. The common white shallot was used in all the tests unless otherwise indicated. Most of the plantings were made in October and the shallots were harvested the following December or January.

APPLICATION OF LIME TO THE SOIL

In 1943, the most heavily infested end of the plot was divided into 12 sections consisting of 6 rows 6 feet wide and 25 feet long. A 5-foot buffer was arranged between each two plots. Four replications of three different treatments were used as follows: A, hydrated lime applied Sept. 15 at the rate of 1,000 lb. per acre; B, lime at 1,500 lb. per acre; and C, check, no treatment. Soil samples were taken from each plot before the lime was broadcast over the plots, and at intervals later. The shallots were planted October 7. The data are in table 4.

White rot first appeared December 5 and was well developed by December 20 when the disease counts were made. At this time the shallots were ready to pull for market. The healthy bunches indicated in table 4 were suitable for market. The shallots were left in the field ten days longer and at this time most of the bunches were diseased. The data show that the lime reduced the amount of early infection. But a few days after maturity the plants in the treated plots were almost as severely affected as those in the untreated plots.

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[4] Personal communication.

TABLE 4.—*The effect of lime on the pH of infested soil, and on the amount of disease in shallots, in 1943*

Amount of lime added to the soil	pH of soil[a]		Total no. of bunches in 4 plots	Number of healthy bunches	Percentage of healthy bunches[b]
	Before lime	After lime			
1000 lb.	5.7	6.4	427	290	68
1500 lb.	5.7	6.6	416	308	74
None	5.7	5.7	414	240	58

a Tests were made 30 days after application of the lime.
b The counts were made Dec. 20, when the shallots were ready to pull.

The same block of infested soil was used in 1944-1945. Additional lime was applied at the rate of 1,500 lb. per acre to two of the lime plots of the previous year as shown in table 5. The lime was applied Sept. 21, and the shallots were planted Oct. 2, 1944. The disease counts and pH readings were made Jan. 3, 1945. The diseased plants were judged by the above ground symptoms alone.[5]

TABLE 5.—*Effect of lime on the pH of infested soil and on the amount of white rot in shallots. Data obtained Jan. 3, 1945*

Section	Amount of lime added to soil		pH of soil	Total no. bunches produced	No. of dead bunches	Percentage of bunches diseased
	1943	1944				
1	1000 lb.	None	5.9	161	4	20
2	None	None	5.5	145	. 2	29
3	1500 lb.	1500 lb.	7.1	158	. 0	6 a
4	1000 lb.	None	5.9	152	2	23
5	None	None	5.4	154	2	22
6	1500 lb.	1500 lb.	7.2	145	0	5 a
7	1000 lb.	None	6.0	156	3	20

a Plots 3 and 6 were in far better condition than the others. Most of the bunches could have been pulled in good condition.

The data in table 5 show that the additional amounts of lime reduced white rot in its early stages. A fairly satisfactory crop of shallots could have been obtained from badly infested soil if the plants had been pulled at the right time. The shallots in this test were left in the field an additional 12 days to see whether or not the lime would keep the disease under control after the plants matured.

The shallots were all pulled and examined Jan. 15. At this time more of the shallots in all the plots were diseased. However, 50 per cent of the bunches in plots 3 and 6 were in a marketable condition, while only 10 to 15 per cent of the plants in the adjoining plots were in good condition.

5 When the plants were pulled, washed, and examined carefully some of the shallots without above ground symptoms were found to be diseased.

This test indicated that the addition of lime sufficient to bring the pH of the soil to about 7 reduced the amount of white rot considerably in the earlier stages of the disease. After the shallots matured sufficiently for the market the leaves died back quickly and the disease made rapid inroads on the weakened plants.

The additional lime applied in 1944 brought the pH of the soil to approximately the neutral point, as is shown in table 5. The plots that received small amounts of lime in 1943 showed little residual effect on the amount of disease in the 1944–1945 crop.

Some of the plots in the block of land used for the liming tests in the 1943 and 1944–45 seasons were used in the 1945–46 season for other purposes. But some information was obtained on the carry-over effects of the lime applied during the two previous seasons. Shallots were planted somewhat later than in the other tests (Oct. 24, 1945). The plants grew more slowly than usual and the white rot showed up later than in any previous season. The shallots were pulled and examined Feb. 13, 1946. At this time the plants had passed their peak of development and had begun to deteriorate somewhat. From plot 1, 12 per cent of the plants were healthy, from plot 3, 30 per cent, and from plot 5, 38 per cent. These figures show little residual effect of the previous applications of lime.

ADDITION OF CHEMICALS TO THE SOIL

Several tests were conducted in which chemicals were applied to infested soil where shallots were planted. Walker (31) found that 138 ml. of mercuric chloride (1–750, 1–1500) per plant applied at transplanting time greatly reduced the incidence of cabbage club root. In our tests the chemical was applied to the soil around the growing plants. A number of seed treatment chemicals were also applied to shallot sets before they were planted in diseased soil.

In the fall of 1943 a preliminary test was conducted in which formaldehyde and Semesan were applied around shallots growing in diseased soil. Formalin (40 per cent commercial) was used in two strengths, 30 and 120 ml. per gallon of water, and Semesan 30 gm. to 1.5 gal. Small plots consisting of single rows 25 feet long were replicated 4 times. The shallots were from 4 to 7 inches tall when the treatments were applied Oct. 7. A small amount of the chemical was poured around each plant, wetting an area 3-4 inches in diameter and about the same depth. The chemical was used at the rate of 1.5 gal. per 100 feet of row. Dry soil was pulled up around the plant to prevent rapid evaporation of the formaldehyde. The formaldehyde caused a severe yellowing of the plants, but none of them was killed. The Semesan caused no apparent injury.

The plants were examined Dec. 20 when they were ready to pull for market. At this time the average percentage white rot for each treatment was as follows: Semesan, 7 per cent; formaldehyde 30 cc. per gal., 27 per

cent, formaldehyde 120 cc. per gal., 16 per cent; and untreated plots, 41 per cent. Two weeks later most of the plants were severely diseased, but in the Semesan plots most of the shallots were in good condition.

In October, 1944, the soil treatment tests were continued in the same plot. Different portions of the plot were used each time in order to prevent after-effects of previous soil treatments. Shallot plants one week old were used in this test. The plants were several inches high and were just beginning to divide when the chemicals were applied (Oct. 9). Semesan (1 oz. per 1 gal. water) and mercuric chloride (1–500) were used at the rate of 80 ml. per plant. An area about 5 inches in diameter and 3-4 inches deep was moistened around each plant. Small replicated plots were used with appropriate controls. The plants were pulled and examined Jan. 15, 1945.

TABLE 6.—*Effect of chemicals applied to soil on white rot of shallot in 1944-1945*

Treatment	Total no. bunches produced	Number of bunches diseased	Number of bunches dead
Semesan, 1 oz. per gal.. .	138	2	0
HgCl$_2$, 1-500 .	137	0	0
Untreated	140	96 a	36

a This figure includes dead bunches.

The data in table 6 show that both chemicals gave almost complete control of white rot under the conditions of this test.

In the fall of 1945, a soil treatment test was made on a slightly larger scale than the previous ones. Mercuric chloride (1–1000) was applied around young shallot plants at the rate of 80 ml. per plant. One block of 6 rows 28 ft. long was treated. The sections on each end of the treated block were left untreated. The chemical was applied Nov. 15, when the plants were 5-7 inches tall. All the plants were pulled and examined for white rot Feb. 13, 1946. The data are in table 7. The mercuric chloride at 1–1000 strength did not give as good control as the 1–500 strength used previously. However, most of the plants in the treated plots held up considerably longer than those in the untreated plots.

TABLE 7.—*Effect of mercuric chloride applied to the soil on white rot of shallot in 1945-46*

Treatment	Total no. of bunches	Number of bunches diseased on		
		Jan. 8	Jan. 30 a	Feb. 13
HgCl$_2$, 1-1000	325	10	50	64
Untreated	331	72	128	184

a Plants ready to pull at this time.

Preliminary tests were made in 1944 with D-D (1, 3-dichloropropene-1,2-dichloropropane) for control of white rot. The D-D was applied to the soil in three concentrations: (1) 5 ml. in holes 6 inches apart; (2) 5 ml. 1 ft. apart; and (3) 15 ml. 1 ft. apart. The holes were 1 inch in diameter and 4-5 inches deep. The D-D was placed in the holes by means of a rubber tube, and the holes were filled with soil immediately. The treatments were applied in small replicated plots. Shallot transplants were set out two weeks after the D-D was applied. Treatments 1 and 2 decreased the amount of white rot appreciably but did not give satisfactory control.

Treatment of seed sets. In the fall of 1944 a small test was made with fungicidal dusts on shallot sets. Barbak D (mercuric phenyl cyanamide) and Arasan (50 per cent tetramethyl thiuramdisulphide) were used. The sets were shaken in paper bags with an excess of the dust, then planted in infested soil. In this test there was no appreciable difference in the amount of disease in the treated and untreated plots. The following year (1945) Arasan was used in a slightly different way. The shallot sets were moistened with water and then coated with Arasan dust before being planted in diseased soil. Again the Arasan failed to give any appreciable control of white rot.

VARIETAL REACTION TO WHITE ROT

Two white shallot varieties make up the bulk of the crop in Louisiana. The common or Chac Bay variety is a small pin-leaf type that has been grown in the State for many years, and the Louisiana Pearl is a large flat leaf type that was bred at the Louisiana Agricultural Experiment Station several years ago for resistance to pink root (24). There are also two red varieties grown in limited areas.

The four shallot varieties or types were grown in infested soil to determine whether there might be some resistance to the disease in any of them. Several shallot seedlings of varying types bred at the Experiment Station in Baton Rouge were also tested for resistance. None of them showed any evidence of resistance to the disease.

DISCUSSION

Walker (30) has shown that white rot of onion is definitely favored by comparatively low soil moisture. He found that the greatest development of the disease occurred when the soil moisture was kept at about 40 per cent of the water-holding capacity. Field observations made in Louisiana show clearly that white rot is much more severe in the higher, better drained portions of the field than in lower, wetter areas. The plants on the ditch bank rows where drainage is best are invariably the most severely affected. In some of the soil treatment tests, the disease was completely inhibited by the treatments in areas 12-15 ft. from the drainage ditch, while on the ditch bank row there was some injury. Factors such as

soil aeration and differences in the physical condition of the soil also may affect the development of the disease.

The matter of dissemination of white rot in this State has been of some interest. One grower whose small farm is most severely affected states that he has had the disease on his place for at least 20 years. No apparent effort has been made to prevent the spread of the disease, but the amount of spread has been remarkably small considering the possible means of dissemination. The writer has been working on one plot of heavily infested soil for four seasons. This plot is separated from an adjoining farm by a head land only 12 ft. wide. Shallots and garlic are grown regularly on the next farm but no white rot has been observed there.

The principal means of dissemination of white rot in shallots here appear to be (1) shallot transplants from diseased areas and (2) the transfer of infested soil by the use of farm implements, on the shoes of laborers, or on the feet of work animals. The disease is not carried through on infested shallot sets to any appreciable extent because they almost invariably rot during the summer. The writer has collected many slightly infected shallot sets for experimental use, but they invariably rotted before they could be used. However, garlic is grown on some shallot farms. Slightly infected garlic bulbs might transfer the causal fungus to new areas.

White rot has proved to be a very difficult disease to control in Louisiana. The addition of lime in sufficient quantity to bring the soil to about pH 7 reduced the amount of disease appreciably but did not give satisfactory control. One year after the lime was applied the disease was almost as severe as before. The use of lime had the same effect as most of the control measures — it only delayed the development of the disease. If the shallots were pulled at exactly the right time the lime, Semesan, and mercuric chloride greatly reduced the severity of the disease. The 1944-45 tests with Semesan and mercuric chloride gave almost complete control because they delayed the development of white rot long enough for the shallots to be harvested. But in most of the treatment tests the plants were killed if they were left in the field very long after maturity.

None of the shallot varieties being grown in the State have any appreciable degree of resistance. However, some shallot breeding is being done for control of other diseases. These progenies will be tested for possible resistance to white rot.

The most promising method for controlling white rot of shallot in Louisiana seems to be early planting. Most of the shallots are planted in September or October. These plants are usually harvested in December or early January. Other plantings are made later during the winter. Often as many as three crops of shallots may be grown on the same soil during one season, beginning about September 1 and lasting until May 1. White rot has not been observed here earlier than about December 1. If

the severely infested areas are planted in late August or early September the shallot crop can be harvested in October or November before the white rot develops to any appreciable extent. Thus one good crop might be harvested from diseased soil each year.

SUMMARY

White rot of shallot has been known in Louisiana only a few years. At present the infested areas are rather small, but the disease is a potential threat to the entire shallot growing industry in the State.

The disease shows as a wilting, yellowing, and dying of the leaves associated with rotting of the roots and basal portions of the shallot plants. Grayish white mycelium and small black sclerotia develop on the underground portions of the affected plants.

White rot is a cool weather disease and develops in Louisiana only during the winter months from December through March. Shallots are grown from September into May, thus exposing them to the disease during a considerable portion of the time that they are in the fields.

The disease is known to affect most of the cultivated species of *Allium*.

Strains of *Sclerotium cepivorum* from shallot were found that varied considerably in color and amounts of mycelium, numbers of sclerotia, and pathogenicity on shallot.

The fungus grows well over a rather wide pH range on media such as Bacto bean agar and Czapek's solution. However, in Richard's solution growth was sharply limited between pH 2 and 7.

Lime applied at the rate of 1,500 lb. per acre reduced the severity of white rot in shallot, but did not give satisfactory control. Semesan and mercuric chloride applied to the soil around growing shallot plants gave fairly good control.

Seed set treatments with a number of fungicidal dusts were ineffective in reducing the amount of disease.

None of the shallot varieties now being grown in the State have any appreciable resistance to white rot.

Early planting of shallots is probably the best means of control in Louisiana. If they are planted early enough (Aug. 20 to Sept. 15), one good crop of shallots can be harvested before white rot develops.

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INFECTIOUS CHLOROSIS OF PHENAX SONNERATII

KARL M. SILBERSCHMIDT[1]

During a survey of species of Malvaceae, attacked by "Infectious Chlorosis," we found a weed, exhibiting similar chlorotic spots, but which belongs to the species *Phenax Sonneratii* (Poir.) Wedd. of the Urticaceae.[2] The first natural habitat of this diseased weed observed by us (on February 3, 1946) was a sandy, shaded place called "Prainha" near the beach of São Vicente (State of São Paulo). Later (April 14, 1947) we collected specimens of *Phenax Sonneratii* exhibiting the same type of symptoms at another sandy spot of the beach of Santos called "Bocaina." On both occasions the leaves of the attacked plants displayed a pronounced veinclearing (Fig. 1, a and b), light green or yellowish angular spots limited largely by the secondary veins (Fig. 1, a).
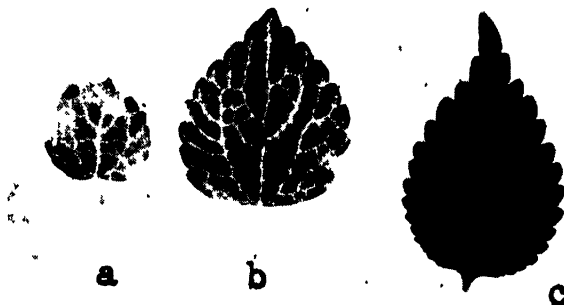
FIG. 1. Leaves of *Phenax Sonneratii*, (a and b) from a plant naturally infected with "Infectious Chlorosis," and (c) from a healthy plant.

In connection with our studies on Infectious Chlorosis of the Malvaceae (9) it was interesting for us to determine if a graft-transmissible disease characterized by chlorotic symptoms could be found also on representatives of the Urticaceae. We brought rooted, diseased plants of *Phenax* from Santos to our Institute in São Paulo, where we grew them in our greenhouse. With this diseased material from the two natural sources we performed the following experiments on the transmissibility of the disease.

On April 1, 1947, four healthy plants of *Phenax* were grafted with diseased scions of the same species. Eighteen days later two of the scions had taken and the respective stocks displayed on their young leaves

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chlorotic symptoms similar to those shown on the scion. Diseased leaves were confined almost entirely to the grafted shoots; other shoots of the same plants continued producing normal green leaves for months.

Other positive results in our attempts to transmit the disease by grafting to healthy plants of the same species were obtained in our experiments of July 7 and August 11. Six out of 18 healthy plants of *Phenax Sonneratii* grafted with chlorotic scions contracted the disease. Healthy test

FIG. 2. Plant of *Phenax Sonneratii*, (a) experimentally infected with "Infectious Chlorosis," (b) a healthy plant of the same species.

plants, grown on the same bench as the grafted ones, and the control plants, which had been grafted with scions of healthy plants or with healthy shoots of diseased plants, never displayed symptoms. Figure 2 shows one of the stocks infected by grafting with "Infectious Chlorosis" beside a healthy control plant.

We did not succeed in transmitting the disease by grafting to *Boehmeria nivea* (Urticaceae) or to *Sida rhombifolia* (Malvaceae).

The seeds collected from diseased sources produced plants without any symptoms of the chlorotic disease (June 3, 1947).

On September 8, we inoculated 7 healthy plants of *Phenax Sonneratii* with sap of diseased shoots, and we rubbed 5 other plants with sap of healthy plants. Until now all these plants have remained without any symptoms of the chlorotic disease.

Returning now to our experiments on the transmission of the disease by grafting, we wished to ascertain if the progress of the disease along a grafted shoot could be speeded up experimentally. Following the method conceived by Hildebrand (6) and used efficiently by Cochran (4) we covered the healthy tip of the grafted shoots with black pasteboard. Taking off the covers after a fortnight, we observed that many of the older shaded leaves had dropped, but that the young, developing leaves displayed symptoms of the disease. The tips of grafted plants, which had not been covered with black pasteboard, remained healthy for a long time.

In view of the characteristic symptoms of this disease and of its transmissibility only by grafting, we consider it a virus disease and suggest it be included in the group of "Infectious Chloroses."

In the literature—as far as we could verify—few virus diseases among representatives of the Urticaceae have been described. As early as 1929 McKinney (7) had observed *Fleurya podocarpa* Wedd. (Urticaceae) displaying yellow mosaic in West Africa. Later, Severin (8) cited *Urtica urens* as host plant of experimentally transmitted sugar-beet curly top. Gardner and Whipple (5) mentioned "nettle" among the experimental host plants of the spotted wilt of tomato. More recently Blattny, Robek, Stary, and Ryzkov in two papers (2, 3) have described a sap-transmissible mosaic disease of *Urtica dioica* and *Urtica urens*, probably the same disease which Blattny (1) had observed much earlier on naturally infected plants of *Urtica dioica*. In none of those publications, however, do the authors refer to a disease of the Urticaceae of the general character of "Infectious Chlorosis" transmissible by grafting, but not by sap inoculation. Therefore, we consider *Phenax Sonneratii* a new host plant of "Infectious Chlorosis."

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FUSARIUM WILT OF GARDEN STOCK (MATHIOLA INCANA)

KENNETH F. BAKER

An important vascular fusarium disease was observed in seed fields of garden stock (*Mathiola incana* R. Br.) and Ten Weeks stock (*M. incana* var. *annua* Voss) in Santa Barbara County, California, during September, 1946. Apparently there has been no published record of a fusarium wilt of this host, but O. A. Plunkett has informed the writer that he observed a similar disease that killed practically all of the plants in a Los Angeles garden about 1928.

Infected plants of the maturing seed crop have characteristic symptoms. The color of the siliques and stems is faded from the usual grayish tan to a light tawny shade (Fig. 1). All or part of the siliques on a stem are flat, as compared with the plump normal condition, and are found to contain flat, undeveloped seeds. There is a somewhat premature shedding of the leaves progressively from the base upward. Any of these symptoms may develop unilaterally. The plants may be stunted and are sometimes killed. Although symptoms at the time of seed maturation are not conspicuous because of normal loss of leaves and green color, it is easy to recognize infected plants once the differences in appearance are noted. There is commonly a brown discoloration of the vascular elements in roots and stems of affected plants. Root cortex apparently does not decay until the plants are dead.

Young plants have conspicuous symptoms within 30 to 40 days after being transplanted into heavily inoculated soil held at about 21°–24° C. Some of the seedlings quickly wilt and die. Older plants may have a striking vein clearing of basal leaves (Fig. 2); this effect is evidenced progressively upward on the stem. Leaves finally turn yellow, but wither before being shed instead of rapidly abscising as in cabbage fusarium yellows. As the plants mature the field symptoms already described appear.

There are several diseases of stock that may be confused with fusarium wilt. Bacterial blight, a vascular wilt disease induced by *Xanthomonas incanae* (Kend. and Baker) Starr *et al.*, causes a characteristic blackening of the leaf scars of the stem (3) not found with the fungus disease, and does not have the tawny color and vein clearing of the latter. Rhizoctonia foot rot, caused by *Rhizoctonia solani* Kühn, girdles the stems of young plants at soil level and causes a gradual or rapid wilting without the tawny color or leaf vein clearing (1); coarse mycelium can be seen with a hand lens on the surface of the stem lesions. Sclerotinia white blight, caused by *Sclerotinia sclerotiorum* (Lib.) Schroet. occurs commonly in seed fields, producing on the flower stalks localized white lesions which girdle the stems; typical large black sclerotia occur in the pith of such stems. Phyto-

phthora foot rot, caused by *Phytophthora cryptogea* Peth. and Laff. and perhaps other parasitic Phycomycetes, decays the roots and crown and results in a wilting of the top; this disease is easily recognized by the

Fig. 1. Ten Weeks stock plants grown in pots in the greenhouse. Plant on left infected with fusarium wilt, showing general stunting and leaf killing, with flattened and light tawny siliques. Plant at right is normal.

soft basal decay (7).   Verticillium wilt of stock is reported from Europe but apparently is unknown in the United States; it might produce symptoms similar to those of fusarium wilt.

The wilt *Fusarium* was readily and consistently cultured from the stems of affected plants gathered in the field.   Siliques from affected stems were surface treated with sodium hypochlorite before being opened, and the seeds, removed aseptically and placed on agar, regularly yielded the same organism.   Commercial seed from these fields also carried this *Fusarium*, along with other fungi.   Pathogenicity for *Mathiola* of isolates from all of these sources was confirmed.   All plants grown in infested soil at 21°–24° C. developed the disease.   Almost all the seed developed on diseased plants 10–12 inches high grown in infested soil in greenhouse pots yielded

Fig. 2.   Lower leaves of column stock plant infected with fusarium wilt.   Normal leaf on left.

the *Fusarium* when placed on agar.   The conditions of growth of these plants precluded the possibility of fungus penetration through the wall of the silique, and indicated that the fungus grew up through the vascular elements.   Not all of these seeds were sufficiently plump to pass through commercial cleaning and milling operations.   The pathogen is clearly seed borne, probably internally.

Pathogenicity of the various isolates was readily demonstrated.   Single-spore cultures were used in all cases and the fungus inoculum was grown on potato-dextrose broth.   The washed mycelial mats were chopped up and

mixed with pasteurized soil in pots into which the stock seedlings were transplanted. The greenhouse chamber was kept at 21°–24° C., and at these temperatures strong symptoms developed. None of the checks was diseased.

The organism was identified by W. C. Snyder as *Fusarium oxysporum* Schl. emend. Sny. and Hans. (6). Two forms of this species, *F. oxysporum* f. *conglutinans* (Wr.) Sny. and Hans. on cabbage and kale (2, 6), and *F. oxysporum* f. *raphani* Kend. and Sny. on radish (4), have been reported on cruciferous crops. Unfortunately, virulent cultures of the kale and radish isolates were not available for test, but a fresh isolate of the cabbage form was obtained. Flats of pasteurized soil were inoculated on April 30, 1947, with mycelial mats of the different forms. The flats were planted with transplants of column stock, Copenhagen Market cabbage, and Dwarf Green Curled kale, and seeds of Early Scarlet Globe radish. Data were taken on June 12. Stocks in the flats with the stock isolates were nearly all dead, but those in flats with the cabbage form were unaffected. Cabbage showed typical yellows with the cabbage *Fusarium*, but no symptoms with the stock isolates. Kale was unaffected by the stock organism, but several of the plants showed typical yellows with the cabbage form. Radish was unaffected by the stock isolates, but showed slight vascular streaking with the cabbage form. All of the positive results reported were confirmed by isolations from vascular tissue. These results indicate that this is a specialized form of *F. oxysporum* which differs biologically from those previously reported on cabbage, kale, and radish. Accordingly, the specialized form which causes vascular wilt of stock, *Mathiola incana*, is designated as *Fusarium oxysporum* f. **mathioli** n.f.[1]

Because a temperature below 18° C. is required for initiation of flower buds of stock (5) the flower crop is grown either under glass or outdoors, depending on the area, during the winter months. Since the vascular fusarium diseases are favored by warm soils (24°–25° C.), it is understandable that this disease has only once been observed in garden or florist plantings, even though the pathogen must have been disseminated commonly with the seed. If a successful summer-flowering stock should be developed, the disease might prove a limiting factor in its use, as has fusarium wilt of China aster.

The California seed crop is sown in the field in December or January, and the seed is harvested the following September or October. Because of this prolonged growth period, the plants mature in the warm months during which the disease appears.

Several different varieties of Ten Weeks, column, and branching stocks have either been observed with the disease in the field or successfully inoculated. It is not known whether any varieties are resistant to this disease.

[1] The spelling follows the correct name of the host, *Mathiola*, rather than the commonly used *Matthiola*. See L. H. Bailey. Hortus, p. 8. Macmillan Co., New York. 1930.

SUMMARY

An important vascular fusarium wilt occurs on maturing stock plants (*Mathiola incana* and *M. incana* var. *annua*) in California seed fields, causing premature leaf drop, stunting, vascular discoloration, and development of flattened, faded, light tawny siliques. In infested warm soil, seedlings have conspicuous vein clearing, stunting, and wilting, and they die early.

The stock *Fusarium* is non-pathogenic to cabbage, kale, and radish, and the cabbage form does not infect *Mathiola*. The pathogen is designated as *Fusarium oxysporum* f. *mathioli* n. f. The organism commonly is seed borne.

Since stock requires low temperatures for bud initiation and is grown in the winter months, the disease may prove to be important only in the seed crop which continues growth through the summer.

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FIELD CONTROL OF BLIND SEED DISEASE OF PERENNIAL RYEGRASS IN OREGON[1]

JOHN R. HARDISON[2]

INTRODUCTION

Blind seed disease of perennial ryegrass (*Lolium perenne* L.) caused by *Phialea temulenta* Prill. and Delacr. was first known to occur in the United States during the winter of 1943. Positive identification was made by Dr. E. O. C. Hyde, Seed Analyst at Palmerston North, New Zealand, who recognized typical macroconidia on Oregon-grown seed sent to him for diagnosis by Mrs. L. A. Kanipe, Seed Analyst in charge of the Federal-State Cooperative Seed Laboratory at Corvallis, Oregon. The first apothecia seen in the United States were found by Mr. O. E. Mikesell, County Agricultural Agent, and a group of farmers on a Linn County farm near Halsey, Oregon, May 12, 1944. Apothecia were first positively identified in Oregon by Dr. George W. Fischer (4, 7).

Preliminary work and observations during the period of the winter of 1943 through the summer of 1944, conducted by Mrs. L. A. Kanipe, Mr. H. H. Rampton, Mr. H. A. Schoth, Dr. George W. Fischer and others, was briefly reported by Fischer (7) and in unpublished reports (4, 5, 6). This preliminary work in Oregon and particularly the published work in New Zealand greatly aided the development of field control in Oregon in connection with the writer's studies which began in September, 1944.

ECONOMIC IMPORTANCE

The detrimental effects of blind seed disease on perennial ryegrass seed make it a very serious disease. Although seeds infected during early development are of light weight and can be mechanically separated, most infected seeds have sufficient weight to prevent their removal from healthy seed in harvesting and cleaning operations (20, 25). There is the further difficulty that infected seed cannot be distinguished easily from healthy seed unless the lemma and palea are removed (14). In germination tests, seed laboratories make no separation of the "blind seeds" from healthy seed. Therefore, severely diseased crops of seed are low in germination and much reduced in value.

Greenall (8) mentions that in New Zealand germinations for two consecutive seasons were as low as one per cent in one case, and in very few

cases higher than 30 per cent. Greenall (8) states further that farmers in the South Otago district of New Zealand may expect low germinations in perennial ryegrass seed one year in every two or three years.

Neill and Hyde (20) state that losses cause high prices of certified seed and thus severely restrict the establishment of new pastures with superior ryegrass from certified lines. They mention further that scarcity and high price of good lines of seed in New Zealand have greatly restricted the development of export trading.
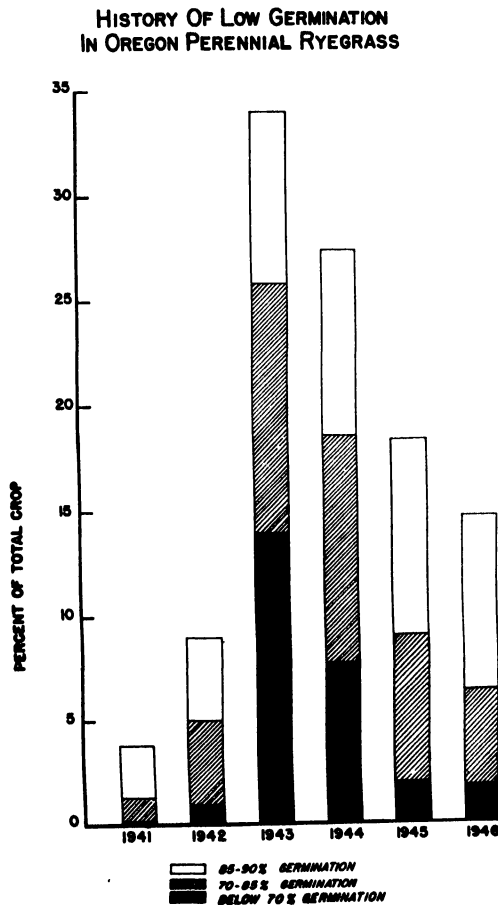
FIG. 1. Bar graph shows history of low germination in Oregon perennial ryegrass Percentages of the total crop are based on individual seed lots.

In Ireland, Calvert and Muskett (1) state that there would be few seasons when by proper blending the seed could not be brought up to a standard of germination suitable for incorporation in mixtures intended for pasture establishment in areas not producing seed. However, this does not prevent severe losses to individual growers.

In Oregon a few cases of low germinating seed appeared in the 1941 crop. Further difficulties were experienced in 1942. The greatest damage occurred in 1943 when one-fourth of the crop could not be certified because of less than 85 per cent germination. Nearly one-seventh of the 1943 crop had less than 70 per cent germination and represented practically unsaleable seed (Fig. 1).

In New Zealand, white clover and perennial ryegrass are often grown as pasture mixtures, and it has been recommended that if the ryegrass seed crop is found badly diseased in preharvest tests the grower may either cut it for hay or perhaps save the field for a later clover seed crop (14, 16). In contrast, perennial ryegrass in Oregon is grown extensively in pure stands primarily for a seed crop; consequently, farmers have no real alternative if the seed crop fails, since returns from ryegrass hay would be only a fraction of that from a good seed crop. Furthermore, a large portion of the perennial ryegrass in Oregon is grown on land that will grow very few other profitable crops. As a consequence, control of blind seed disease is of considerable importance to Oregon agriculture.

<div align="center">LITERATURE REVIEW</div>

Wilson et al. (25) review the literature concerning the identity and nomenclature of the blind seed disease fungus. The many contributions to the biology and control of blind seed disease by previous workers may be summarized as follows:

1. Blind seed disease is caused by *Phialea temulenta* Prill. and Delacr., and this is apparently the same fungus previously ·described from rye (*Secale cereale* L.) in France by Prillieux and Delacroix (22) in 1892 (1, 21, 25).

2. The fungus overwinters in infected seed, producing apothecia during the spring coincident with the flowering of the grass (1, 20, 21, 25).

3. Ascospores initiate the infection, which is followed by a copious production of macroconidia in a slimy matrix on infected seeds that may infect additional flowers (1, 20, 25). Macroconidia produced in artificial culture are equally infectious (20).

4. The seed is the only part of the plant infected, and therefore presence of disease in seed does not affect the value of the grass for pasture, cover crops, or turf (1, 20, 25).

5. Endosperm tissue is permeated by the fungus mycelium and the embryo may often be killed (12, 20, 25).

6. Macroconidia die within a few weeks after harvest and are of no importance in perpetuating the fungus during or after storage of seeds (21).

7. Mycelium in infected seeds dies in natural dry storage within 20 to 24 months (20).

8. The fungus within seeds has been killed by dry heat (21), and by

hot water (1, 2), and partial control has been obtained by the use of chemical dusts (2, 25), but a practical method of seed treatment has not yet been discovered.

9. Preliminary trials indicated that deeper sowing suppresses, at least in part, the development of apothecia, and drill sowing was recommended in preference to broadcasting (25).

10. The disease spreads considerably by air-borne ascospores (1, 20).

11. Perennial ryegrass seeds infected with blind seed disease are non-toxic to animals (21).

12. Methods for examining preharvest head samples and harvested seed samples for the presence of the disease have been described (6, 7, 14, 15, 16, 24, 25).

13. The control suggestions in New Zealand (21) and in Scotland (25) consist primarily as follow: (a) examining crops for infestation before harvest to enable growers to avoid expense of harvesting worthless crops, and (b) planting two-year-old seed or seed from healthy crops.

14. The available control measures have not given satisfactory field control (3, 8).

MATERIALS AND METHODS

A method for rapid determination of the incidence of disease in seed samples, which is a modification of the New Zealand system and the method previously reported by Fischer (7), was described in an unpublished report by Fischer (6). This consisted of mixing equal parts of seed and water, approximately 30 ml. of each, and allowing the seed to soak for about 30 minutes, with occasional shaking, after which the water was separated from the seeds by straining through cheesecloth. A drop of this liquid was then applied to a haemacytometer, and the conidia of the blind seed disease fungus within a specific size division of the counting cell were counted.

In the present studies, this method has been used with only slight modification. Glass vials of approximately 18-ml. capacity are labeled with the seed sample number, and used to measure the seed which is placed together with the same amount of water into a numbered 250-ml. Erlenmeyer flask. These same vials are retained to receive the water strained from the seed after soaking for 20 minutes. A Bausch and Lomb haemacytometer with Neubauer ruling is used to standardize the method. By comparison with the haemacytometer, ordinary microscope slides and cover glasses were found to give reliable results that can be easily interpolated to the haemacytometer readings, if the cover glass is firmly pressed to the slide and care is taken to avoid excess water.

Rose (24) used a similar method by heating 100 seeds in a test tube with 5 ml. water for 15 min. at 80° C., after which the tubes were shaken for half a minute. A drop of the solution was then taken for a conidial count with a Zeiss Thoma haemacytometer and the number covering the 16 large

squares registered. Rose (24) concluded that the method cannot be used for harvest forecast purposes, and he gave preference to Hyde's (14) method of examining individual seeds for infection.

In the Oregon program current harvest forecasts have been largely discontinued, and the method now used appears to be satisfactory for advising growers of the possibilities of the next year's crop based on the probable increase in disease and consequent seed damage.

Since it has been necessary to indicate the severity of disease in seed samples to growers in ordinary terms, the classification shown in table 1 was arbitrarily designed. In this paper all references to degree of infection are based on this classification, which has functioned satisfactorily and is still being used.

TABLE 1.—*Classes of infection for blind seed disease of perennial ryegrass based on tests of cleaned seed samples*

Name of class	Macroconidia per 1/160 cu. mm. [a]	Two-year average germination for c'ass	Field recommendation for next crop
	Number	*Per cent*	
1. None	0	93.0	Safe for harvest
2. Trace	1–3	92.5	Safe for harvest
3. Very Light ...	4–6	91.5	Should plow if wet field
4. Light	7–15	90.0	Should plow
5. Moderate	16–30	87.0	Plow
6. Moderately Heavy.	30–50	79.0	Plow
7. Heavy	50–100	76.5	Plow
8. Very Heavy	100–200	65.5	Plow
9. Extremely Heavy. ..	200–600 +	57.0	Plow

[a] Number of macroconidia recovered in water suspension after soaking seed 20 minutes in an equal volume of water.

EXPERIMENTAL RESULTS

Seed Treatment Studies

Results of seed treatment trials planted by H. H. Rampton and H. A. Schoth during the fall of 1943 were reported by Fischer (4, 7). These tests gave no practical control by chemicals or dry heat. Partial control was indicated by a vapor heat treatment conducted for these workers by Miller and McWhorter in the manner they have described (18, 19).

In further trials the writer planted plots in the fall of both 1944 and 1945, using heavily diseased seed treated with proprietary chemicals including Arasan (tetramethyl thiuram disulfide), New Improved Ceresan (ethyl mercury phosphate), Semesan (hydroxy mercury chlorophenol), Spergon (tetrachlorobenzoquinone), Purex (sodium hypochlorite), Yellow Cuprocide (yellow cuprous oxide), and liquid formaldehyde. No reduction in numbers of resulting apothecia was noted. The dry heat treatment as described by Neill and Hyde (21) gave a 65 per cent reduction in apothecia with only a slight reduction in germination. A vapor heat treatment per-

formed by Miller and McWhorter (19) consisting of an approach period of 20 min. at 130° F. followed by 30 min. at 145° F., gave complete control of the fungus with no reduction in germination.

Because sufficient disease-free seed and two-year-old seed have been available to meet the needs of growers for planting new fields for seed production, seed treatment is regarded as impractical in Oregon, and further trials have not been made.

Aging of Seed

Neill and Hyde (21) first reported that no apothecia were obtained from two-year-old seed. Based on this information two-year-old seed was recommended to Oregon growers during the winter of 1943 in a report prepared by H. H. Rampton and Mrs. L. A. Kanipe (23).

The writer planted two-year-old seed in the fall of 1944 and 1945. None of this seed produced apothecia the following spring. On the other hand, in two years' trials, diseased seed, three and 15 months old, planted in the fall (October) produced abundant apothecia the next spring.

The first suggestion of control by spring planting was mentioned by Neill and Hyde (20) and later suggested by Fischer (4, 7). However, observations were incomplete, since observations were made only during the same calendar year diseased seed was planted and not after the seed had been exposed to winter temperatures.

In the writer's studies diseased seed 10 months old planted in the spring (April and May) has produced abundant apothecia the following spring in amounts equal to that obtained from fall planted trials with the same seed. In contrast, samples of heavily diseased seed 22 months old planted in May, 1945, produced no apothecia in 1946.

Based on the above information, Oregon seed growers have been advised that diseased seed is safe for fall planting when past 24 months old (9, 10, 11) and for spring planting when 20 to 22 months old (10, 11).

Depth of Planting Studies

Wilson et al. (25) mentioned that experimental evidence indicated that deep planting at least partially suppressed apothecia arising from the seed planted. In the writer's trials during the winter of 1944, seeds buried one-fourth inch or more in moist sand in laboratory tests produced no apothecia, although apothecia were abundant on surface seeds. A field planting was made in October, 1945, with an ordinary grain drill. The soil had been summer fallowed and permitted excellent coverage of seed. No apothecia were obtained in the spring of 1946 from seed buried one-half inch or more.

One farmer in Linn County inadvertently drilled heavily diseased, 1942 crop seed (68 per cent germination) at a depth of 2½ to 3 inches during September, 1943. The field produced two excellent subsequent crops, 97

per cent germination with a trace of disease in 1944, and 95 per cent germination with a very light infection in 1945. There can be little doubt that the accidental deep planting was responsible for the small amount of disease in this instance.

Planting seed at least one-half inch deep or more with complete soil coverage has been recommended in Oregon as an additional safeguard (10, 11).

Chemical Soil Treatments

Fischer (5) suggested the application of chemicals to suppress apothecia in infested fields. In May, 1945, the writer applied 24 different chemical treatments to plots in a heavily infested field near Peoria, Oregon. The treatments included the following chemicals as sprays: Special Semesan (hydroxymercurichlorophenol and hydroxymercuricresol), mercuric chloride and mercurous chloride (1 : 2 mixture), copper sulfate, copper oxide, basic copper sulfate, Sinox (sodium dinitro ortho cresylate), basic Sinox (Sinox plus sodium hydroxide), Puraturf (phenyl mercuri triethanol ammonium lactate), sodium arsenate, Isothan Q15 (lauryl isoquinolinium bromide), and lime-sulfur. Chemicals applied in dry form were Calcium cyanamid (calcium cyanamide), dicyandiamide, sulfur, crude naphthalene, and Dithane A-10 (sodium ethylene bisdithiocarbamate).

Most of these chemicals injured apothecia which were present at the time of application; however, no chemical was effective for more than a short period. Calcium Cyanamid at 150 lb. per acre completely suppressed the apothecia for three days, but normal apothecia were abundant after eight days. The other treatments gave no control or had not completely suppressed apothecia three days after application, and abundant apothecia were noted after eight days. Considering that perennial ryegrass plants are in anthesis over a period of several weeks, these soil treatments did not have practical value. Further trials were omitted because records for the 1945 crop indicated that control had been obtained from the program in operation (Fig. 1).

Trials with Head Sprays and Dusts

During the summer of 1945, flowering spikes of perennial ryegrass in a heavily infested field were given a single application with seven sprays: Puraturf, Isothan Q15, Dithane D-14 plus zinc sulfate and lime (sodium ethylene bisdithiocarbamate), Fermate (ferric dimethyl dithiocarbamate), Sinox-D (ammonium dinitro ortho cresylate), lime sulfur, and Bordeaux mixture 5–5–50, and two dusts: sulfur and Sinox (15 per cent dinitro ortho cresylate). These materials were applied May 25, 1945, when a maximum number of flowers were in anthesis. No reduction in amount of disease was noted for any of the treatments, and further trials have not been made. Corkill and Rose (3) also reported no value from spraying ryegrass plants during the infection stage with various chemical sprays.

Burning as a Control Measure

Perennial ryegrass fields are sometimes burned to remove excessive straw left after combine seed harvesting. One 600-acre field located in Linn County, Oregon, which in 1944 produced a heavily infected crop with 48 per cent average germination and two years' straw accumulation, was burned after harvest in August, 1944. After burning, only a few seeds could be found in the entire field. In the spring of 1945 apothecia were present only in a small area that had been poorly burned. The 1945 crop showed only a trace to very light amount of disease with an average of 97 per cent germination. Three other diseased fields with germinations between 73 and 93 per cent in 1944 were burned after harvest. All three produced seed that germinated above 90 per cent in 1945.

A comparison of the results from burning, in table 2, with the records on similar fields that were not burned, shown in table 3, definitely indicates that burning will reduce blind seed disease and subsequent damage to seed crops. The variation in the reduction of disease shown by the four fields in table 2 is due primarily to the differences in the effectiveness of different fires. The intensity of the burning as evidenced by the completeness of removal of crop residue was best in field A and in decreasing degrees in the order D, B, and C.

TABLE 2.—*Effect of burning straw and stubble of perennial ryegrass, after the 1944 harvest, on blind seed disease and seed germination in the 1945 crop*

Field	Acres in field	1944 crop (before burning)		1945 crop (after burning)	
		Average germination	Amount of disease	Average germination	Amount of disease
	Number	*Per cent*		*Per cent*	
A[a]	618	48	Extremely Heavy [b]	96	Very light
B	212	73	Very Heavy	95	Light
C	200	90	Moderate	95	Light
D	12	93	Light	94	Trace

[a] Field had two years' straw accumulation resulting in a hot, clean burn.
[b] See Classes of infection described under Materials and Methods.

Burning as a remedial control measure is recommended in Oregon only for fields with a marginal infestation after the first or second year's crop. Older fields generally become weedy and seed yields drop. It has been recommended (10, 11) that such fields be plowed to clean up the field and to save the organic matter in the crop residues in preference to burning.

Preharvest Tests for Disease

The principal control measure in foreign countries is the testing of preharvest samples for disease so that growers can avoid harvesting worthless

crops. Unfortunately this system does not insure the farmer a return
from his land as far as seed is concerned, and, more important, it leaves
heavily infested fields as sources of abundant inoculum for continued infec-
tion in old fields and possible infestation of new fields.

Preharvest testing was started in Oregon during the summer of 1944
under the auspices of Federal-State Cooperative Seed Laboratory at Cor-
vallis, with the help of Dr. G. W. Fischer. At first the New Zealand
method described by Hyde (14) was used, but Fischer (4, 6, 7) developed
a faster method relying on the number of macroconidia recovered from
soaking chopped up heads to indicate severity of infection. At first (4, 7)
a centrifuge was used to separate the conidia, but later (6) a haemacyto-
meter was used to count the conidia in the water residue.

Preharvest testing was discontinued as a regular practice in Oregon
during the fall of 1944 and was replaced by the seed testing service.

Absence of Disease in Regrowth Heads

In 1945 and 1946, samples of regrowth heads which developed in heav-
ily infested fields after harvest were carefully examined. No blind seed
disease has been found in regrowth heads. This indicates that no increase
of disease potential occurs in a field after the regular harvest.

Oregon Program for Seed Inspection and Field Recommendation

Since the control measures proposed by other workers failed to provide
satisfactory field control according to Greenall (8), further measures were
thought necessary to control the disease in Oregon. The most apparent
weakness in foreign control programs appeared to be the failure to make
some disposition of badly diseased fields. Therefore, during the winter of
1944, the writer set up a program for examination of seed samples from
every perennial ryegrass seed production field in Oregon entered for
certification. A portion of the bulk seed from the certification samples
was made available for the disease tests. From the disease tests reports
were prepared for all growers, listing the amount of disease found and
giving recommendations for field control.

The problem immediately arose to determine the degree of infection
in seed samples that would make a field unprofitable for another year's seed
production. It was learned from case histories that seed from many fields
had dropped from above 90 per cent germination to below 85 per cent in
the next crop. Table 3 shows the records for two seed crops from nine
perennial ryegrass fields. These records were selected as typical examples
from the available case histories and illustrate the approximate increment
in disease that may be expected in an infested area. From these records
it is apparent that the disease can build up in a field, without noticeably
reducing germination, to a point where severe damage will result the
following year.

After conducting numerous tests for incidence of disease and comparing the results with the germination of respective seed samples, the eight classes of infection described under Materials and Methods were designed to classify individual samples. The degree of infection in seed samples that would result in a germination below 85 per cent in the next crop was estimated to be the Light class of infection (Table 1). Fields with more disease, *viz.*, Moderate, Heavy, Very Heavy, or Extremely Heavy infestations were regarded as unprofitable for another year, and the recommendation was made (9, 10) that such fields be plowed before May 1, since apothecial production begins in early May. Fortunately this recommendation was rather generally followed. Growers who did not follow these directions invariably produced low quality seed during 1945 and 1946. The plowing of a majority of the badly infested fields has prevented direct loss to growers concerned and has greatly reduced the inoculum available for infestation of new fields. This is especially important, since most new fields have been planted with either disease-free or two-year-old seed since the fall of 1944.

TABLE 3.—*Increment of blind seed disease in nine selected perennial ryegrass fields with different degrees of infection. Fields located in Linn County, Oregon*

Field	1944 crop		1945 crop	
	Percentage germination	Disease infestation	Percentage germination	Disease infestation
A	93	None seen	95	Trace
B	96	Trace	92	Very Light
C	95	Trace	90	Light
D	95	Very Light	90	Moderate
E	91	Light	85	Heavy
F	91	Light	81	Heavy
G	92	Light	76	Very Heavy
H	85	Moderate	72	Extremely Heavy
I	88	Moderate	38	Extremely Heavy

For the fields having a Light amount of disease in the seed test, the information given was that the next year's crop would be a gamble, and that the field should be plowed. It was explained to growers (9, 10) that they would profit by cleaning up such fields and returning them to production of high quality seed as early as possible instead of taking a chance of heavy loss for the year; and also that badly infested fields would constitute serious hazards to neighboring fields in the meantime. The majority of growers with such fields decided to plow them, especially after the 1945 crop results had indicated general success from the recommendations (Fig. 1).

As shown in table 1, perennial ryegrass seed may have a germination of 90 per cent or higher, yet may have considerable blind seed disease infection. Therefore, fields that have produced high germination seed cannot necessarily be regarded as safe, since such fields may have a Light

or Moderate disease infestation and may produce a crop of low germination seed the following year. A spectacular example was a field near Halsey, Oregon, that produced seed of 90 per cent germination but with a Light disease infestation in 1944. The 1945 crop had an average germination of 38 per cent with an Extremely Heavy infection of blind seed disease.

Fields with no disease, a Trace, or a Very Light amount of disease were regarded at first as probably safe for another year's production. Although many fields with a Very Light infestation have produced seed the next year that averaged well above 90 per cent germination, a number of fields on wet lands with this degree of infestation have produced seed of poor germinability. As a result the recommendations have been modified, and it is now suggested that fields with a Very Light infestation be plowed if the field is poorly drained and likely to be wet in May and early June.

In making recommendations to County Agricultural Agents, it is suggested that, when advising growers, they should give attention to local conditions, such as age of fields, presence or absence of infested adjoining fields, burning, and other factors that might influence the expected disease infestation.

During the three years the program has been in operation, there has been a gradual elimination of diseased fields, and an increase in fields without disease. In the 1946 crop 52 per cent of the fields had no disease as compared with 27 per cent in 1945 and 13 per cent in 1944. If this trend continues, the disease eventually may be almost completely eliminated as a limiting factor in seed production of perennial ryegrass in Oregon.

Disease-Free Seed Stocks

A valuable by-product of the examination of all samples of Oregon perennial ryegrass is information that makes possible the selection of disease-free seed lots for planting new seed producing fields. Seed lots having no disease, 95 per cent germination, and blue tag purity have been selected and approved from each crop starting with 1944. This has provided growers with a source of officially approved seed and has contributed materially to the success of the control program. The supply of such seed was limited in 1944 but greatly increased in 1945. In 1946, more seed stock of this quality was available than was needed for reseeding. Similarly Calvert and Muskett (1), have recommended that the best seed be kept for distribution in seed-producing areas of Ireland.

Evidence of Control

The data illustrated by the bar graph in figure 1 indicate that good control of blind seed disease in Oregon was obtained in 1945 and 1946. The improvement for the 1944 crop in comparison with 1943 appears to have been due in part to dryer weather conditions. Also, farmers plowed most fields that produced low germination crops in 1943. In addition, some

attention was given to planting high quality seed in the fall of 1943. A substantial part of the 1944 crop was cut for hay and not harvested for seed because of the recommendations from the Experiment Station based on preharvest tests. This reduced the amount of badly diseased seed entered for certification, and consequently affected the data in figure 1. The improvement in the 1945 and 1946 crops appears to be justifiably credited to the control program put into practice during the winter of 1944. Using 1943 as a year very favorable for blind seed disease, a comparison of weather records for the Willamette Valley indicates that 1944 was relatively unfavorable and that 1945 and 1946, while less favorable than 1943, were much more favorable than 1944 for blind seed disease devolopment. The large amount of badly diseased seed produced in the unfavorable 1944 crop year suggests that the decided improvement in Oregon perennial ryegrass in 1945 and 1946 should be credited primarily to the control program described in this paper.

The control measures that are recommended in Oregon (9, 10, 11), may be summarized as follows:

1. Plant disease-free seed approved by the Experiment Station.
2. Recommendations on planting diseased seed:
    A. Diseased seed is safe for fall planting only when more than two years (25 to 27 months) old. Seed 15 months old is as dangerous as that three months old when fall planted.
    B. Spring planting of diseased seed is safe when seed is 20 to 22 months old. Diseased seed from the previous harvest, 9 to 11 months old, is not safe for spring planting.

3. Plant seed in all seed production fields at least one-half inch deep with complete soil coverage as an extra margin of safety. Good seedbed preparation facilitates this practice.

4. Plow up badly infested fields when notified by the Experiment Station. Plowing should be done before May 1 to prevent emergence of apothecia and consequent danger to nearby fields. ·

5. Preharvest tests are justified only for those fields indicated as marginal in the seed test.

6. Cultural practices that will reduce the disease are as follows: Pastures may be a source of infestation; therefore, it is recommended that pastures should be planted with disease-free or two-year-old seed, and that no heading in pastures be permitted until after July in seed producing districts. Clean plowing is desirable to bury the blind seeds below the furrow slice if possible. Good soil drainage would be helpful, since apothecial production is favored by wet soils.

DISCUSSION

Blind seed disease of perennial ryegrass has been successfully controlled in Oregon by the control program described in this paper. A comparison

of the Oregon program with programs in foreign countries suggests that one of the main reasons for lack of control elsewhere has been the failure to eliminate infested fields. Because of the natural spread of the disease by ascospores, the excellent control measure consisting of planting disease-free or properly aged seed cannot provide more than partial control when the disease is left unchecked in nearby fields. This situation is acknowledged by Neill and Hyde (20) who suggest the development of resistant varieties as the most hopeful method for control. The situation, as it appeared in 1943 the world over, was well described by Greenall (8) who stated that much is known about the disease but little about its control. Corkill and Rose (3), as late as 1945, reported that none of the suggested control methods appeared to be completely effective in controlling the disease in a season favorable for disease development.

Since there is a fundamental difference between culture of perennial ryegrass in Oregon and in foreign countries, it may not be practical to operate elsewhere the plan as developed in Oregon. In foreign countries perennial ryegrass apparently is grown primarily for pasture, with seed production a secondary proposition. Greenall (8) states that seed production in New Zealand depends on wet weather furnishing abundant pasture so that part of the plantings can be closed up for seed production. In contrast, in seed harvest areas of Oregon perennial ryegrass is grown primarily for seed production, and pasture use of seed fields, while important, is a secondary utilization. This striking difference makes it easy to put into operation the plan described under the Oregon program and probably would make it difficult to operate in foreign countries. The plowing up of infested but otherwise good forage producing fields would appear to be difficult to obtain in New Zealand, especially when the risk of seed production is not too good.

Under these circumstances, it is difficult to determine how the disease could be controlled satisfactorily in other countries unless by resistant varieties or by designating special districts for seed production and others for forage as suggested by Hyde (12).

Hyde (12) suggested that the more arid districts must be looked to for quality seed production in New Zealand. This may be a good plan. It is noticeable that the disease has been slow to penetrate into the drier areas in Oregon. This, however, probably will not be an important factor in Oregon because most of the ryegrass seed is produced and probably will continue to be produced on land with poor drainage, making it very favorable for disease development. Climatic conditions appear to favor the disease nearly every year over a large part of the seed production area.

The pre-harvest testing plan for control as practiced in foreign countries is not adapted to Oregon conditions and was discarded early in these investigations. This plan which involves the detection and cutting of worthless crops for hay or waiting for a later clover seed crop invites difficulties

the following year, since this procedure leaves the maximum amount of diseased seed in a field. The disease potential is very high, and if soil moisture is sufficient to permit production of apothecia, the crop may be a total loss. Since in New Zealand (8) much of the seed production depends on abundant rainfall, the production of good quality seed is always jeopardized.

Under the Oregon program for inspection of seed samples, farmers are advised months in advance of the next harvest whether or not they can expect a good crop. Worthless crops can be avoided and infested stands destroyed long before harvest, permitting the farmer to produce other crops or fallow his land. At the same time, other fields are protected by this removal of heavy inoculum that would otherwise be disseminated. The owners of safe fields have increased assurance of good crops. This program has nearly stabilized the perennial ryegrass seed producing industry in Oregon as far as seed germination is concerned.

In controlling this disease, consideration must be given to the fact that the production of seed of perennial ryegrass, in common with that for most other forage crops, normally returns a relatively low acre income. Therefore, most of the control to be practical must be derived through cultural practices. Fortunately, the successful control program in Oregon consists largely of proper cultural practices with close attention to the developmental history of disease in each field. The service to growers, consisting of seed inspection and field recommendation, is in demand and is being adopted as a function of the Oregon Experiment Station.

Seed treatment tests failed to provide satisfactory control in Oregon. This substantiates findings elsewhere. However, ample amounts of aged seed and disease-free seed have been available to meet the needs of Oregon farmers for planting new seed production fields.

Trials on suppression of apothecia and protective head treatments did not meet with practical success. This approach to the problem is regarded as impractical, and further trials have not been made.

Planting seed deep to prevent emergence of apothecia, as first suggested by Wilson *et al.* (25), has met with general success in both laboratory and field trials and has been further verified by field observations. Planting seed in all new seed fields at least one-half inch deep with complete soil coverage has been recommended in Oregon for 1945 and 1946 (9, 10) as an extra precaution against spread of the disease.

Burning stubble and straw residue has given good control in badly infested fields. However, in Oregon, burning is regarded as a drastic treatment and is recommended only for fields that have become severely infested during the first or second year. It is suggested that older fields be plowed because yields are generally declining and weeds are usually becoming a serious problem (9, 10).

The disease has not been found in regrowth heads that invariably form after harvest in perennial ryegrass fields. This suggests that there is no increase of the disease in a field after the regular seed harvest in Oregon.

The investigations in Oregon have been in large part, by necessity, a matter of field experimentation on a very large scale. By a study of case histories it was possible to predict fairly well the course of events in specific fields. The program involving recommendations to farmers is still subject to improvement and probably will be further refined to meet changing conditions. The success so far attained suggests that the program is worthy of trial in other countries.

SUMMARY

1. Blind seed disease of perennial ryegrass has been controlled successfully, apparently for the first time, in fields in Oregon. Control is based on the elimination of badly diseased fields through a program of inspection of seed samples from the previous crop from all fields and recommendations developed for growers.

2. Experimental work indicated the following:

Seed treatment, protective head sprays and dusts, and chemical soil treatments to suppress apothecia are not of practical value.

Aging of seed eliminates danger of spread by seed.

Seed planted one-half inch or deeper with complete soil coverage prevented emergence of apothecia.

Burning straw and stubble after harvest controlled the disease for at least one year.

The disease has not been found in regrowth heads produced after the regular seed harvest.

3. Preharvest testing as a control measure is regarded as impractical in Oregon and has been replaced by the program of inspection of seed samples.

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BOOK REVIEWS

WOLF, FREDERICK TAYLOR. *The Aquatic Oomycetes of Wisconsin*. Part 1. 64 pp, 52 fig. The University of Wisconsin Press, Madison, Wisconsin, 1944. $1.50.

In the present confused state of fungus classification and nomenclature, the determination of species remains a difficult task requiring the attention of specialists in particular groups. The task is brought within the range of the general mycologist, however, with the appearance of local floras of this type, in which the literature is organized, nomenclatural problems are reduced, and the work of identification simplified because of the limited number of species to be considered. A local flora is of value only to the extent that it is based upon critical study of a sufficient amount of material to assure a reasonable degree of completeness. Wolf's study of fifty-five species in twenty-two genera of the Lagenidiales, Blastocladiales, Monoblepharidales, Saprolegniales, Leptomitales, and Pythiales appears to be satisfactory in this respect. It includes several species of pathological importance. The descriptions and illustrations of species and the keys to facilitate their identification should be a welcome aid to mycologists and pathologists in Wisconsin and adjacent areas, while the introductory discussion and the extensive list of literature should find even wider interest. More specific information on the extent of the survey upon which the work is based would make an estimate of its probable completeness more precise. Also, a general discussion of habitats and ecological relationships might be a desirable addition. There must inevitably be omissions in an initial survey of such obscure organisms. Nevertheless, the work is an important contribution and stimulus to the production of a comprehensive flora.—E. S. LUTTRELL, Department of Botany, University of Missouri, Columbia, Missouri.

DICKSON, JAMES G. *Diseases of field crops*. 401 pp. McGraw-Hill Book Company, Inc. New York and London. 1947. $4.50.

This book presents briefly and concisely the important information on diseases of field crops. It is a revision of "Outline of Diseases of Cereal and Forage Crop Plants of the Northern Part of the United States," published in mimeoprint by Burgess Publishing Company, Minneapolis, in 1939. There has been considerable change in the scope of material presented: diseases of five additional crops have been included, significant new information has been added, and the literature list for each chapter has been revised and brought up to date. In addition, 102 illustrations have been included.

The text is divided into four sections: Part 1, General introduction, includes a brief discussion on physiologic anatomy of certain plants in relation to diseases; Part 2, Diseases of cereals and grasses; Part 3, Diseases of legumes; and Part 4, Diseases of fiber and other field crops. In addition to a good index, the book also contains an appendix in which the diseases are grouped on the basis of the primary causal agents and a second appendix in which the causal organisms are arranged according to order and family.

The crop plants are arranged alphabetically in each section. As in the previous publication, the diseases of each crop are considered in the following sequence: non-parasitic, virus, bacterial, phycomycetous, ascomycetous, hypomycetous, and basidiomycetous diseases. Although detailed discussion of a particular disease is given under one crop only, considerable repetition sometimes appears if the disease occurs on several hosts. This also necessitates frequent cross-references to different chapters. If the book were organized on the basis of diseases, its value as a text book would be materially increased. This would allow for more definite information on certain diseases without increasing the volume of the book. However, the book was intended as a convenient reference outline and I presume many crop specialists would prefer the arrangement on the crop basis.

The more important diseases are discussed from the following standpoints: Geographic distribution, economic importance, symptoms, causal agent (and morphology of the organism), conditions conducive to development, and control measures; while less important diseases usually are presented briefly. Keys for the determination of physiologic races of many of the important pathogens are included.

Many of the illustrations in the book are excellent and valuable for diagnostic purposes, others are poorly reproduced, and a few are worthless. The quality of illustrations probably may be attributed to the type of paper used, and I presume will be remedied as soon as a better quality of paper becomes available. The book is well written and relatively free from typographical errors. The book should be of material value to pathologists and crop specialists everywhere. The author should be commended not only for bringing a vast amount of material together in one volume, but because of his generosity in relinquishing all claims to royalties and thus reducing the price of the book.—J. J. CHRISTENSEN, University Farm, St. Paul 1, Minnesota.

HOSTS OF THE TOBACCO STREAK VIRUS[1]

ROBERT W. FULTON

The tobacco streak disease was first described, from Wisconsin, by Johnson (7). He reported it in 1935 as usually occurring on scattered plants, having seen it in abundance only once. Since then it has become much more common and has been reported from other states and countries. It is widely distributed in Wisconsin; by the middle of early August nearly every field of tobacco has at least a few infected plants. A high percentage of infection along the borders of fields is not uncommon.

In order to devise control methods, information is needed on the manner in which the virus overwinters and how it spreads to tobacco. The localization of the disease near the edges of tobacco fields led Johnson (7) to suggest that infected perennial weeds were the source of the virus. Except for the experimental infection of 35 species of *Nicotiana* by Diachun and Valleau (6), only a few species in other genera are known to be susceptible to the virus. Anderson (2) reported the infection of jimson weed and ground cherry and thought that the virus probably was transmitted from these hosts to tobacco. A high incidence of tobacco streak near perennial sweet-clover has been reported by Valleau (8) and Berkeley and Phillips (4). In Wisconsin, none of the localized areas of heavily infected tobacco has been found adjacent to sweetclover. For this reason, other plants were suspected of harboring the virus. The present paper reports various plant species found infected near tobacco fields, and lists species found susceptible by artificial inoculation.

METHODS AND MATERIALS

To determine the incidence of the streak virus in wild and crop plants, collections were made in areas near infected tobacco fields. Young leaves of plants showing virus-like symptoms, as well as others appearing healthy, were macerated in a mortar and the extract wiped with a gauze pad on the leaves of 5 small healthy Havana-Seed tobacco plants in the greenhouse. The tobacco leaves were dusted lightly with carborundum before being inoculated, and rinsed with water after inoculation. These plants were observed for two weeks or longer under conditions favorable for the development of streak symptoms.

In determining the experimental host range of the virus, species[2] of plants, selected at random, were grown from seed in the greenhouse. When

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still young, the plants were dusted with carborundum and inoculated with a fresh extract of the streak virus from recently infected tobacco plants. After 10 to 14 days, an attempt was made to transfer the virus from these plants to 5 healthy tobacco plants. If no infection resulted, the original plants were reinoculated and a second effort was made to recover the virus on tobacco.

NATURAL INFECTION IN WEEDS AND CROP PLANTS

In the late summer and fall of 1946, 159 inoculations were made from various species collected on 6 farms, on 5 of which tobacco streak was

FIG. 1. Leaves of burdock (*Arctium minus*). showing the toothed type of malformation due to infection with the tobacco streak virus. The leaf on the left is healthy; the leaf on the right is from a naturally infected plant.

abundant. Nine of these inoculations produced typical tobacco streak on tobacco. Table 1 shows the species found infected and the number of times that the virus was obtained from each. As a check on the identity of the viruses isolated, it was shown that "recovered" leaves of the infected tobacco plants did not develop new symptoms when inoculated with known tobacco streak virus.

The symptoms on these naturally infected plants are inconspicuous,

except on burdock (*Arctium minus* (Hill) Bernh.). The leaves of infected burdock plants are strikingly toothed and thickened, with prominent light colored veins (Fig. 1). The infected plants appear very similar to an anonymous description (1) of *Arctium minus* form *laciniatum*. This taxonomic form may have been based on plants infected with the streak virus.

The survey was continued in the spring of 1947 in the same localities. Tobacco streak was recovered 12 times in 127 inoculations from various plants. The virus was most consistently isolated from burdock (Table 1).

TABLE 1.—*Plant species collected during 2 seasons and found to be naturally infected with the tobacco streak virus*

Species	Farm	Collected fall, 1946		Collected spring, 1947	
		Plants tested	Plants infected	Plants tested	Plants infected
		Number	*Number*	*Number*	*Number*
Burdock	A	9	2	7	3
(*Arctium minus*	B	3	0	3	2
(Hill) Bernh.)	C	1	0		
	D	...	......	3	2
	E	..	..	5	0
	F			2	1
Hedge Mustard	A	5	3		
(*Sisymbrium*	B	1	1		.
officinale (L.)	D	1	0		.
Scop.)					
White Clover	A	3	2	4	1
(*Trifolium*	C	4	0		.
repens L.)	D			4	0
	E	1	0	1	0
	F	1	0	1	0
Bindweed	A	4	1		
(*Convolvulus*					
arvensis L.)					
Plantain	A	1	0	4	0
(*Plantago*	D	2	0	1	1
major L.)	E	1	0	3	0
	F		..	3	0

This may have been due to the conspicuous symptoms, which made it easy to recognize infected plants. On two occasions, not listed in table 1, infected burdock plants were found some distance away from tobacco fields. The virus was never recovered from burdock showing mosaic, yellowing, necrosis, or symptoms other than the toothed type of malformation. Burdock, in its perennial habit and its wide and abundant distribution, fulfills all the requirements for an overwintering host of the streak virus.

Twenty-four inoculations were made from sweetclover showing various virus-like symptoms. None of these produced tobacco streak. Valleau (8) and Berkeley and Phillips (4) have reported difficulty in mechanically

transmitting this virus from sweetclover to tobacco. For this reason, negative evidence may be of little significance. The virus could be transmitted readily, however, from artificially infected sweetclover in the greenhouse, indicating that at least some infection might have been obtained from field grown plants if they had contained the virus.

EXPERIMENTAL HOST RANGE

Since the species of plants found naturally infected, including tobacco, represented 6 different families, it seemed likely that the virus might be able to infect many others. In greenhouse tests, this proved to be true. Of the 169 species selected at random and inoculated, the virus was subsequently recovered on tobacco from the following 87 species, listed by families. Species in parentheses were not tested but are listed because they have been reported susceptible by other authors.

APOCYNACEAE
Vinca rosea L.

ASCLEPIADACEAE
Asclepias syriaca L.

CARYOPHYLLACEAE
Cerastium vulgatum L.
Lychnis alba Mill.
Silene noctiflora L.
Spergula arvensis L.

CHENOPODIACEAE
Beta vulgaris L.
Chenopodium album L.
Spinacia oleracea L.

COMPOSITAE
Arctium minus (Hill) Bernh.
Callestephus chinensis Nees.[3]
(Calendula officinalis L.)[4]
Ambrosia artemisifolia L.
A. trifida L.
Cichorium endiva L.
Taraxacum officinale Weber
Sonchus oleraceus L.
Lactuca sativa L.
Venidium fastuosum
Zinnia elegans Jacq.

CONVOLVULACEAE
Convolvulus arvensis L.
Convolvulus sp.

CRUCIFERAE
Brassica arvensis L.
Iodanthus pinnatifidus (Michx.) Steud.
Sisymbrium officinale (L.) Scop.

CUCURBITACEAE
Cucumis sativus L.
C. melo L.
Cucurbita maxima Duchesne.
C. pepo L.
Citrullus vulgaris Schrad.

Sicyos angulatus L.

LABIATAE
Nepeta cataria L.

LEGUMINOSAE
Aeschynomene indica L.
Crotalaria intermedia Kotschy
Cyamopsis tetragonalobus (L.) Taub.
Dolichos biflorus L.
Hedysarum coronarium L.
Melilotus alba Desr.
M. officinalis Lam.
Phaseolus aconitifolius Jacq.
P. aureus Roxb.
P. vulgaris L.
Pisum sativum L.
Psoralea bituminosa L.
Robinia pseudo-acacia L.
Soya max (L.) Piper.
Trifolium glomeratum L.
T. incarnatum L.
T. praetense L.
T. repens L.
Trigonella sp.
Vicia faba L.

MALVACEAE
Abutilon theophrasti Medic.
Althaea rosea L.
Hibiscus esculentus L.
Hibiscus sp.
Gossypium hirsutum L.
Malva rotundifolia L.

NYCTAGINACEAE
Mirabilis jalapa L.

ONAGRACEAE
Oenothera biennis L.

PLANTAGINACEAE
Plantago major L.

POLYGONACEAE
Fagopyrum esculentum Gaertn.

Rumex britannica L.
R. crispus L.
Polygonum persicarae L.

PORTULACACEAE

Portulaca.oleracea L.

ROSACEAE

Fragaria virginiana Duchesne.
Geum sp.
Potentilla monspeliensis L.

SOLANACEAE

Capsicum frutescens L.[3]
Datura metel L.
D. stramonium L.[4], [5], [6]
Lycium halimifolium Mill.
Lycopersicon esculentum Mill.[7], [8]
Nicandra physaloides (L.) Pers.[4], [5]
Nicotiana spp.

Petunia hybrida Vilm.[8]
Physalis pubescens L.[5], [6]
Schizanthus wisetonensis Low.
Solanum aculeatissimum Jacq.
S. dulcamara L.
S. integrifolium Poir.
S. melongena L.[7]
S. nigrum L.
S. triflorum Nutt.
S. tuberosum L.[7], [8]

SCROPHULARIACEAE

(Antirrhinum majus L.)[4]
Verbascum thapsus L.

TROPAEOLACEAE

Tropaeolum majus L.

UMBELLIFERAE

Apium graveolans L.

Under high greenhouse temperatures (80° F.) and short-day conditions, the following symptoms were characteristic of the families involved: *COMPOSITAE*, mosaic or necrotic flecking; *CUCURBITACEAE*, yellow mottle; *LEGUMINOSAE*, necrosis, slight mottle, or symptomless; *MALVACEAE*, brown necrotic spots and lines; *ROSACEAE*, mosaic and stunting; *SOLANACEAE*, chlorotic ring spot, mosaic, or systemic necrotic lines. On most of the other species the symptoms were not distinctive.

All of the species listed were infected by mechanical inoculation except *Pisum sativum* L. and *Trifolium praetense* L. These were infected through a dodder (*Cuscuta campestris* L.) connection to diseased tobacco, the virus being transferred mechanically back to tobacco after removal of the dodder.

Ring-spot symptoms were produced on *Phytolacca decandra* L., and necrotic flecking on *Cichorium Intybus* L., but no virus could be recovered from these species either mechanically or through dodder.

The infection of 3 rosaceous species, and the subsequent recovery of the virus on tobacco, are of some interest. Bawden and Kleczkowski (3) have pointed out that no virus infecting the Rosaceae is known to be transmitted mechanically. They have suggested that this is due to virus inactivation by tannin when the infected leaves are crushed. Apparently this is not the case with the tobacco streak virus. Thirty-four of 40 tobacco plants became infected when inoculated with leaf extracts from infected Rosaceae, but only 12 of 44 rosaceous plants became infected when inoculated with the streak virus from tobacco. The limiting factor here appeared to be the relative insusceptibility of the 3 species.

A number of other species also showed a high degree of resistance. None of the cruciferous species could be infected consistently, and several solan-

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[3] Previously reported not susceptible by Berkeley and Phillips (4).
[4] Previously reported susceptible by Berkeley and Phillips (4).
[5] Previously reported susceptible by Johnson (7).
[6] Previously reported susceptible by Anderson (2).
[7] Previously reported not susceptible by Johnson (7).
[8] Previously reported susceptible by Valleau (9).

aceous species, particularly tomato and potato, could not be infected regularly.

The species which did not become infected are not listed, since in some cases only a few plants were inoculated. It is likely that at least some of these would prove susceptible if larger numbers were used.

QUANTITATIVE ESTIMATION OF VIRUS INFECTIVITY

One of the purposes of inoculating a large number of species was to find a host more suitable than tobacco for certain types of research. A species
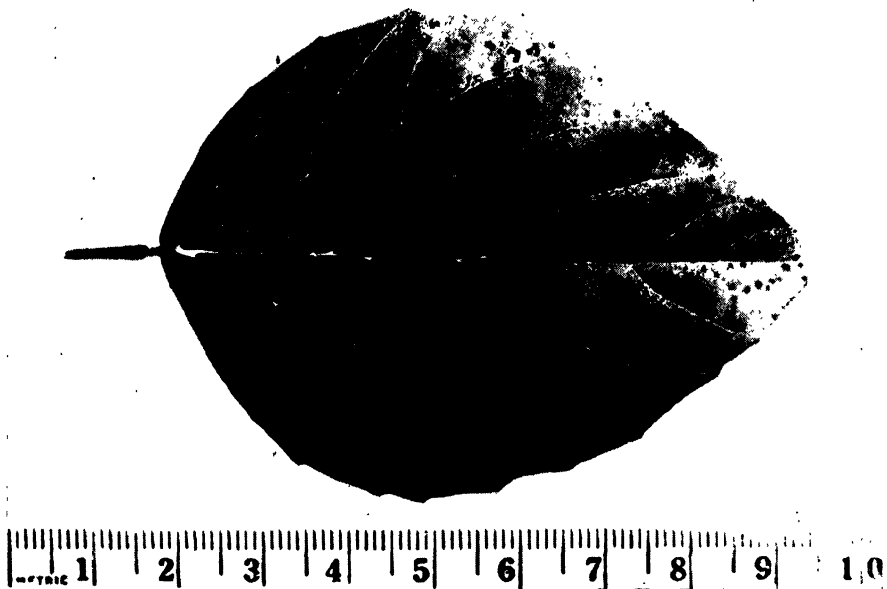
FIG. 2. Local lesions produced by the tobacco streak virus on guar (*Cyamopsis tetragonalobus*). The leaf was inoculated with a 1:1 dilution of the streak virus, and photographed 3 days after inoculation. The scale shown is in centimeters.

which develops local lesions would be particularly useful. Some varieties of garden beans developed the diffuse, reddish-brown, necrotic spots described by Berkeley and Phillips (4). Others, principally selections of the Great Northern variety, developed distinct necrotic spots. The number of lesions was small, and occasionally no infection could be obtained. Another legume, guar (*Cyamopsis tetragonalobus* (L.) Taub.), was much more susceptible than bean. Small, dark, local lesions appeared about 48 hours after inoculation (Fig. 2). These showed little tendency to enlarge or spread, remaining distinct for 10 days or more. The first 3 or 4 leaves the plant produces are simple and usually 5 to 8 cm. long. Although the leaves are not as large as the primary leaves of bean, the lesions were small, and several hundred on one leaf could be accurately counted.

Since an inoculum of high infectivity is necessary for consistent infection, some measurements of virus infectivity on guar are presented. As has been reported by Diachun (5), the presence of $M/10$ Na_2HPO_4 in the inoculum increased the number of lesions. It was found further that the amount of infective virus in tobacco was much greater in the inoculated leaves than in other parts of the plant. Comparisons of infectivity were made using inocula prepared by grinding 0.1 gram of tissue in 0.4 ml. of $M/10$ Na_2HPO_4. Three different extracts were inoculated in pairs on opposite half-leaves of guar, so that each possible pair occurred once on each of 3 leaf positions. Thus each extract was inoculated to a total of 6 half-leaves on 3 guar plants. In one trial, with inoculum taken from a tobacco plant which had been infected for 10 days, the extract of the inoculated tobacco leaves gave a total of 132 lesions on 6 half-leaves of guar, while extracts of the systemically diseased, and the symptomless tobacco leaves gave totals of 5 and 1 lesions, respectively. Other trials gave similar results.

It was also found that a marked decrease in infectivity occurred in extracts within a few minutes after the infected tissue was ground. In one representative trial, the left halves of 3 leaves on 3 plants were inoculated immediately after grinding 0.1 gram of tissue in 0.9 ml. of $M/10$ Na_2HPO_4. The right halves were inoculated, in a Latin square design, after 5, 10, and 15 minutes. The leaves inoculated immediately gave an average of 95 lesions on 3 half-leaves. The total lesions on the 3 half-leaves inoculated after 5, 10, and 15 minutes were 88, 62, and 31 respectively. Other trials, extending over longer periods, showed a greater decrease in the number of lesions. In order to obtain highly infective inoculum, therefore, tobacco leaves which had been inoculated 5 to 10 days previously were ground with $M/10$ Na_2HPO_4 and used immediately. Many of the host plants could be infected only when this practice was followed.

<div style="text-align:center">DISCUSSION</div>

The recovery of the tobacco streak virus from various species near tobacco fields in the late summer, and again the following spring, indicates the manner in which the virus overwinters. In view of the wide host range, there undoubtedly were other species infected which were not found. The evidence indicates that burdock is an important reservoir for the streak virus in Wisconsin. It may be that in other regions different species are more commonly infected.

The relative importance of the different susceptible species to the streak disease of tobacco is difficult to determine. In the absence of a known insect vector, the actual spread of the virus from a wild host remains to be demonstrated. The habits and feeding preferences of the vector will determine, to some extent, which host plants are most important to the disease on tobacco.

The evidence suggests that the disease is endemic in certain wild plants. It is difficult, however, to account for the increasing amount of the disease on tobacco. Each of the 5 species found naturally infected is common and widely distributed. The virus is evidently not limited in its distribution by a lack of susceptible hosts.

SUMMARY

The following plants were found naturally infected with the tobacco streak virus in the vicinity of diseased tobacco fields: Burdock, white clover, hedge mustard, bindweed, and plantain. Burdock appears to be the weed which is most commonly infected with streak in Wisconsin.

A large experimental host range was found for the virus, 87 out of the 169 species inoculated being susceptible. Inoculum prepared from previously inoculated tobacco leaves was much more infective than inoculum from systemically diseased, or upper, symptomless tobacco leaves. The infectivity of the inoculum decreased rapidly within 15 minutes after the tissue was ground.

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THE YELLOW-NET VIRUS DISEASE OF SUGAR BEETS[1]

EDWARD S. SYLVESTER [2]

INTRODUCTION

An apparently undescribed virus disease of the sugar beet (*Beta vulgaris* L.), causing a severe veinal chlorosis (Fig. 1, right) and transmissible by the green peach aphid, *Myzus persicae* (Sulzer) was collected in the field in June, 1945.[3] The virus is not juice transmissible, but can be transmitted with ease by means of the vector. The aphid retains the ability to transmit the virus throughout its experimental life, and consequently the virus is considered to be a persistent aphid-borne virus (24).

REVIEW OF LITERATURE

The previously described virus diseases of sugar beets are: curly top (21), Argentine curly top (2), sugar beet leaf-crinkle (26), savoy (4), beet mosaic (9), beet virus yellows (10), sugar-beet yellow vein (1), yellow wilt of sugar beets (3), and cucumber mosaic in sugar beets (9).

The yellow-net disease differs from all of these diseases in its syndrome. In addition, it varies from curly top, Argentine curly top, beet leaf-crinkle, savoy, yellow vein, and yellow wilt in that it can be transmitted by an aphid.

The three additional aphid transmissible virus diseases of sugar beets, *i.e.*, beet mosaic, beet yellows, and cucumber mosaic, have been recognized in the literature for a number of years. The sugar beet mosaic disease is world wide in its distribution (7, 11, 13, 14, 16). This virus is juice transmissible and is not retained for more than a few hours by its vector (23). The symptoms consist of an initial vein-translucency or yellowing on the youngest leaves. This is succeeded by a diffuse light and dark green mottling over the entire plant. Initial mild leaf distortion may occur in severe cases. In mature leaves the symptom expressed is usually one of small light green rings on a dark green background. The early stage of vein yellowing in some of the mosaic specimens often is indistinguishable from that produced by the yellow-net virus. However, the later phases of the two diseases are distinct in symptomatology.

Sugar beets are susceptible to the cucumber mosaic virus. This virus, like the beet mosaic virus, is juice transmissible, and is not retained by the aphid vectors.

The symptoms induced in sugar beets by the cucumber mosaic virus are local lesions on inoculated leaves. These lesions are yellow, have diffuse margins, and tend to enlarge as the leaf ages. In systemic infection, the newly developing leaves are affected with a partial chlorosis, often apical, which is not confined by tissue regions. There is no vein translucency or vein yellowing in the newly developing leaves. Later the leaves develop a coarse green and yellow mottle, and at times the green areas cup outward to produce blister-like elevations. There is stunting and leaf malformation. In symptomatology, juice transmissibility, and vector relationships, this virus disease differs clearly from the yellow-net disease.

FIG. 1. Leaves from sugar beets experimentally infected with the yellow-net virus showing the chlorosis of the veins and veinlets.

The beet yellows virus and the yellow-net virus have two characteristics in common. They are transmitted by aphids and they are not juice inoculable. Beet yellows has been noted in England (14) and Europe (16), but not in the United States. Symptomatologically, the two diseases are distinct. Hull and Watson (10) reported the symptoms of beet yellows as consisting of a yellowing, thickening, and a brittleness of the outer or middle-aged leaves of the affected plant. The young central leaves generally remain green, and under favorable growing conditions they make moderate growth. Usually, however, in an early infection, growth almost ceases. The yellowed leaves later become necrotic and die. The symptoms

produced by the yellows virus are distinct from those induced by the yellow-net virus, and it is unlikely that any confusion between the two diseases would arise.

Bennett (1) made note of a disease of sugar beets which he termed sugar-beet yellow vein. He stated that the virus disease had not been described to date, but had been known to occur since 1936 in experimental plots at Riverside, California. It also had been known to occur in Colorado. The symptoms reported consisted of a "distinct yellowing along the main veins of leaves, dwarfing of the plant (often more marked on one side), and general yellowing of the foliage." Bennett also stated that the virus was not juice transmissible, but had been transmitted by grafting, and "apparently the virus is not transmissible by the aphid, *Myzus persicae* (Sulz.)."

The author has discussed the symptoms of the sugar-beet yellow vein disease with Bennett and has seen photographs of diseased plants. From the symptoms expressed on the photographed leaf blades, it would be difficult to distinguish the disease produced by the sugar-beet yellow vein virus and that induced by the yellow-net virus. However, the stunting, often asymmetrical, exhibited by plants infected with the sugar-beet yellow vein virus has never been observed in connection with the yellow-net virus. Furthermore, the latter virus is readily transmitted by the green peach aphid.

In spite of the similarity of the leaf symptoms of the two diseases, it is considered that the presence of asymmetrical stunting and lack of aphid transmission in the yellow vein disease described by Bennett is of sufficient importance to permit separation of that disease from the yellow net disease described in this paper.

MATERIALS AND METHODS

The original diseased plants were obtained on Sherman Island near Rio Vista, California. In later experiments, plants inoculated from these original plants were used as virus sources.

Noninfective aphid colonies were maintained upon large healthy sugar beets and originated from a colony of green peach aphid feeding upon a disease-free beet plant in the greenhouse.

The test sugar beet plants were grown from seed which had been planted in flats and later transplanted into 4-inch pots. The plants were grown, inoculated, and incubated for the appearance of symptoms in the greenhouse.

The aphids were transferred from plant to plant with a camel's-hair brush. The plants, during the feeding periods of the vectors, were caged in the standard type of cage described by Severin *et al.* (6, 22). Following inoculation, the plants were usually fumigated with nicotine to kill the insects present.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Yellow-net of sugar beets has been noted in the field only in northern California. Leach has sent specimens from Sherman Island and Isleton, California. Specimens have been collected near Mt. Eden, California, and in the Rio Vista area. Thus far, diseased specimens found in the field have been limited in number and relatively isolated.

SYMPTOMS

The symptoms manifested in a typical specimen of the disease consist of a yellow network of veins and veinlets against a background of green

FIG. 2. Leaves from Swiss chard experimentally infected with the yellow-net virus showing variations in the magnitude and intensity of symptom expression.

interveinal tissue. The symptoms may appear within 9 days following inoculation of a small healthy beet seedling by means of an infective aphid. Usually the first symptoms are expressed on the youngest leaf of the plant. When the youngest leaf exhibits symptoms early in its development, the main lateral veins arising from the midrib often are the only ones affected by the chlorosis. As the leaf expands and matures, the chlorosis involves more and more of the veinlet network. It is from such symptoms that the name, yellow net, is derived.

Variation from the typical symptom pattern is of common occurrence, but it appears to be merely a difference in intensity of symptom development, both in the number of veins and veinlets affected and in the lateral dispersion of the chlorosis from the veinlets into the adjacent tissues.

Frequently one of the first recognizable symptoms found in an inoculated plant consists of one to several scattered yellow spots on a leaf blade (Fig. 2, left). The number, shape, and size of the spots vary, but the usual shape is circular and the usual diameter is approximately 1 mm. Occasionally, only one spot can be detected upon an entire plant.

Usually, following the appearance of the yellow spot phase, the yellow-net phase develops. The extent of involvement of the veinlet network varies, *i.e.*, in some cases only some of the larger secondary veins and larger veinlets are affected (Fig. 2, left), while in other instances it appears as if every vein and veinlet has been affected by the chlorosis (Fig. 2, center).

In some plants the chlorosis involves more area, by diffusing from the veinlets into adjacent mesophyll areas. This diffusion produces a pattern of wide yellow veins separated from one another by the green mesophyll tissue. The lateral diffusion may increase and the areas of spreading chlorosis coalesce, thus surrounding small areas of green tissue and producing a green island pattern. In severe cases this phase is followed by complete obliteration of the green islands of tissue by further diffusion of the chlorosis, and the leaf blade then is completely chlorotic. The color of the chlorosis may vary from cream to golden yellow under greenhouse conditions. The complete chlorosis is confined to the oldest leaves, while the younger leaves of such plants usually have a rather broad, diffuse form of the yellow-net symptom.

In older inoculated plants, the symptom expression may be confined to a yellow spot phase or a slight peripheral yellow net pattern. In some cases, as the plants grow older, the symptoms tend to become milder, or even disappear. Virus recovery trials from such plants have demonstrated that virus is still present, and that it will induce typical symptoms.

Other variations are those in which only certain portions of the leaf blade are affected. One of the more common examples of this type is an asymmetrical symptom expression by a leaf blade (Fig. 2, right). In other cases only the base of the leaf blade may be affected, while in others symptoms may be limited to one of the lower quarters of the blade. These variations probably are associated with the state of maturity of various portions of the leaf blade when invasion by the virus occurs.

The type of symptom expression and the apparent inability of the virus to be transmitted mechanically suggest that the virus is dependent upon the phloem for successful inoculation. Since the symptoms, at times, tend to fade in older plants, it would seem likely that the chlorosis is due in part to interference with plastid development, much as in the case of some other virus diseases (5).

APHID TRANSMISSION

The first diseased specimens were received in June, 1945. Initial juice transmission trials were negative. The diseased plants were potted and

1 month, at the end of which time a colony of noninfective green peach aphid was placed upon each plant. Following the completion of a 10-day feeding period, the aphids from each plant were transferred to a series of 5 healthy beet seedlings for 3 days. The beet mosaic virus was recovered only in the first series, and the yellow-net virus was recovered not only in the first series, but also in the series of each succeeding day. Table 2 gives the results in detail.

A second group of sugar beets, infected with the yellow-net virus and exhibiting good symptoms, was inoculated with the curly top virus by means of 5 infective male *Eutettix tenellus* (Baker). Four beets were inoculated, and following an incubation period of 3 to 4 weeks, all exhibited

TABLE 2.—*Results of trials to recover virus from sugar beets infected with both the yellow-net virus and the sugar-beet mosaic virus by means of previously noninfective green peach aphid apterae fed upon the infected beets 10 days and then transferred daily to lots of 5 healthy beet seedlings.*

Virus source beet no.	Virus recovered	Number of beets infected on three successive days			Total infected out of 15 inoculated
		1	2	3	
1	Mosaic	0	0	0	0
	Yellow net	2	3	5	10
2	Mosaic	0	0	0	0
	Yellow net	2	2	1	5
·3	Mosaic	4	0	0	4
	Yellow net	3	3	1	7
4	Mosaic	0	0	0	0
	Yellow net	3	4	3	10
5	Mosaic	0	0	0	0
	Yellow net	4	5	3	12

symptoms of curly top on the newly developing leaves, again not accompanied by the symptoms of yellow net. After about 3 months, a noninfective colony of green peach aphid was introduced on to each of the 4 sugar beets, and 15 days later the aphids were transferred from each of the 4 beets to a series of 5 healthy beet seedlings. The yellow-net virus was recovered from each of the 4 sugar beets which was infected with the combination of viruses. Following the completion of this test, noninfective males and nymphs of the sugar-beet leafhopper were fed on the 4 infected beets, and 24 hours later were transferred from each of the 4 plants to 2 healthy beet seedlings. In this manner the curly top virus was recovered from each of the 4 beets.

In both instances of combination infection, the established virus, the yellow-net virus, was replaced in symptom expression upon the newly developing leaves by the introduced virus, either the sugar-beet mosaic virus or the curly top virus. This condition remained in effect for 3 to 4 months, after which time some of the plants exhibited symptoms of both viruses. In

any event, whether the plants exhibited a combination of symptoms, or only those of the secondarily introduced virus, the primary virus (yellow net) could be recovered by means of previously noninfective aphids, and recovered in a form which produced symptoms of typical pattern and severity in the recipient healthy beet seedlings.

Numerous workers (8, 12, 15, 25) frequently have used insects to separate combinations of viruses in plants.

From these tests, it is felt that all three of the sugar beet viruses used are distinct.

HOST RANGE

The following varieties of sugar and garden beets were tested and found susceptible to the yellow-net virus: U.S. 15, U.S. 22 (improved), U.S. 23, U.S. 33, G.W. 49, Detroit Dark Red, and Early Wonder. Swiss chard, *Beta vulgaris* L., var. *cicla* Hort., (Fig. 2) is susceptible to the virus, and manifests typical yellow net symptoms.

The following species of plants failed to develop symptoms when inoculated with the yellow-net virus.

Chenopodiaceae: *Atriplex patula* L., Spear orache; *A. rosea* L., Red orache; *Chenopodium murale* L., Nettle-leaf goose-foot; *Spinacia oleracea* L., Spinach.

Compositae: *Calendula officinalis* L., Pot marigold; *Callistephus chinensis* Nees, China aster; *Lactuca sativa* L., var. *romana* Hort., Romain lettuce; *Sonchus arvensis* L., Sow thistle.

Convolvulaceae: *Ipomoea purpurea* Roth, Morning glory.

Cruciferae: *Brassica campestris* (L.), Common yellow mustard; *B. chinensis* Baily, Pak-choi; *Capsella bursa-pastoris* (L.), Shepherd's purse.

Cucurbitaceae: *Cucumis sativus* L., Cucumber.

Geraniaceae: *Erodium moschantum* L'Her., White-stem filaree.

Plantaginaceae: *Plantago major* L., Common plantain.

Polygonaceae: *Polygonum coccineum* Muhl., Swamp knotweed; *P. persicaria* L., Lady's thumb; *Rumex crispus* L., Curly dock.

Solanaceae: *Datura stramonium* L., Jimson weed; *Lycopersicon esculentum* L., Tomato; *Nicotiana tabacum* L., Turkish tobacco.

SUMMARY

Yellow net, a previously unreported virus disease of the sugar beet, *Beta vulgaris* L., is described. The main symptom of the disease is a bright yellow chlorosis of the veins and veinlets of infected plants. The virus is not mechanically transmissible.

The green peach aphid, *Myzus persicae* (Sulzer) transmits the virus. The insect, after becoming infective, remains so for its life under experimental conditions.

The average incubation period of the virus in the sugar beet is 24 days,

with a minimum of 9 days. The virus may become available to a previously noninfective vector 8 days following inoculation.

Sugar beets can be infected simultaneously with yellow-net and beet-mosaic viruses, each in a recoverable state by insects, and the same is true of yellow net and curly top.

The host range of the virus appears to be limited, but all varieties of sugar beets and garden beets tested are susceptible to infection, as is Swiss chard.

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RHIZOCTONIA CAROTAE N. SP. AND GLIOCLADIUM AUREUM N. SP., TWO NEW ROOT PATHOGENS OF CARROTS IN COLD STORAGE[1]

WM. E. RADER

Surveys of stored carrots made during 1943–1946 have shown some sixteen diseases to be present in cold and common storages in New York State. Two of these diseases were found to be caused by pathogens which are believed to be new. These new pathogens which occur in both mineral and muckland soils may cause damage during storage in some seasons amounting to 5 or 10 per cent of the crop from certain fields. They are described here for the first time under the names Rhizoctonia crater rot and Gliocladium rot.

RHIZOCTONIA CRATER ROT

Ramsey (7) reported in 1934 what is apparently the same disease as the one discussed here, although he gave no complete description of the organism. Specimens were shipped to him by one of the state inspectors at Rochester, New York. In 1942 the disease was brought to the attention of the staff at the Cornell Agricultural Experiment Station by the Fairport Storage Company. The disease apparently had been noted previously, but during that storage season losses were severe. During the next two years losses were light; however, again in the storage season of 1945–1946 the disease was found by the writer in epiphytotic form in several cold storages in New York State. At Albion, New York, by January 1, 1946 it was estimated that 10 per cent of the roots had lesions caused by this pathogen. In a cold storage at Fairport, New York, an average of 4 per cent of the roots were affected, with certain blocks running considerably higher. In one block of carrots in a cold storage at Williamson, New York, an estimated 5 per cent of the roots were affected.

Since the causal organism can flourish at temperatures below those maintained in cold storages, this disease may reach epiphytotic proportions and cause heavy losses in cold storages provided the relative humidities are high.

Symptomatology

Rhizoctonia crater rot has been found only on the roots which have

been kept in cold storage for two to three months. No visible symptoms are evident at harvest time. This disease is first evident when small, whitish hyphal knots of the pathogen appear on the roots (Fig. 1, A). Small pits
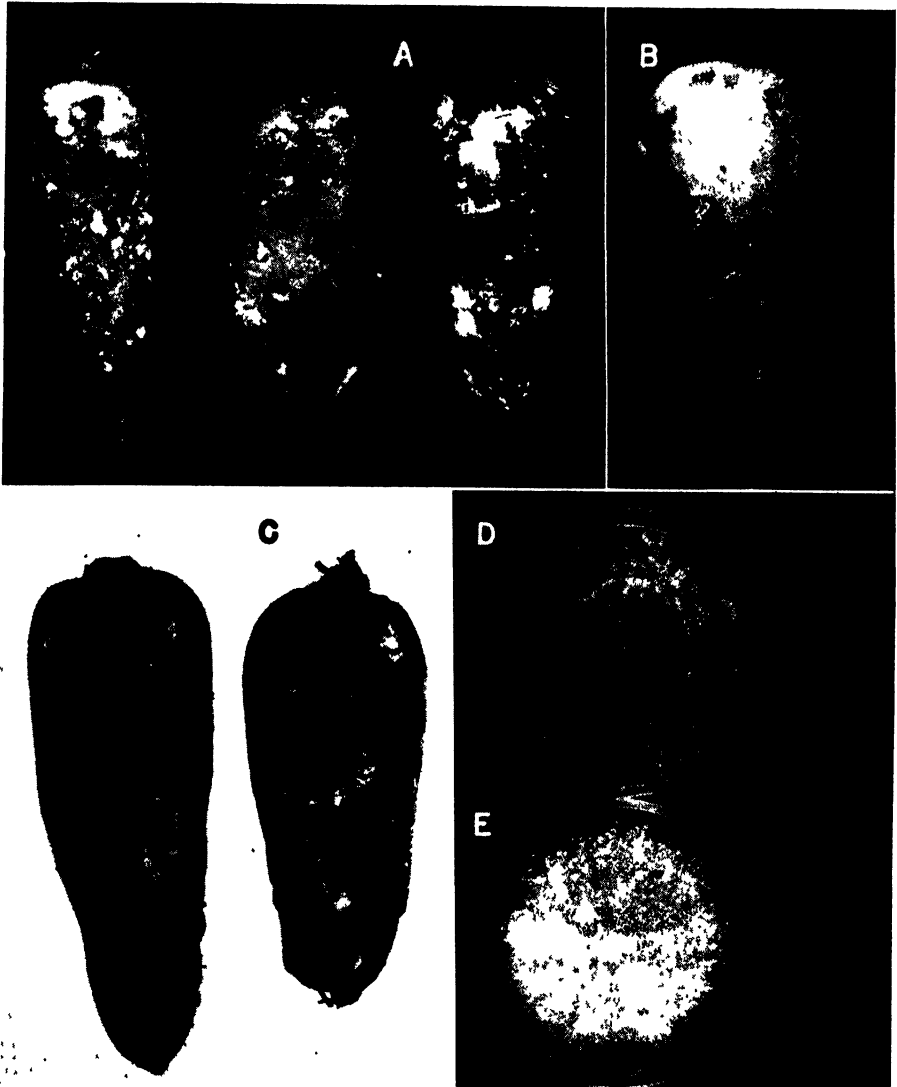
FIG. 1. Crater rot of carrots. (A) early stages of the disease on carrot roots, (B) cankers produced by artificial inoculation, (C) typical ''craters'' produced in the advanced stages of the disease, (D) culture of *Rhizoctonia carotae*, and (E) culture of *R. solani*, both incubated 18 days at 15° C. on potato-dextrose agar.

then appear under these hyphal knots. At this stage the disease can readily be confused with either the Fusarium dry rot, *Fusarium roseum* (Lk.) emend. Snyder and Hansen (*F. avenaceum* (Fr.) *Sacc.*), or the Glioclad-ium rot, *Gliocladium aureum*, described later. In many cases positive diag-

nosis of the cause of these small pits can only be made with the aid of a microscope.   The characteristic mycelium of the pathogen can be readily identified.   The pits enlarge into sunken craters lined with a white flocculent mycelium (Fig. 1, C).   Because of these characteristic craters the common
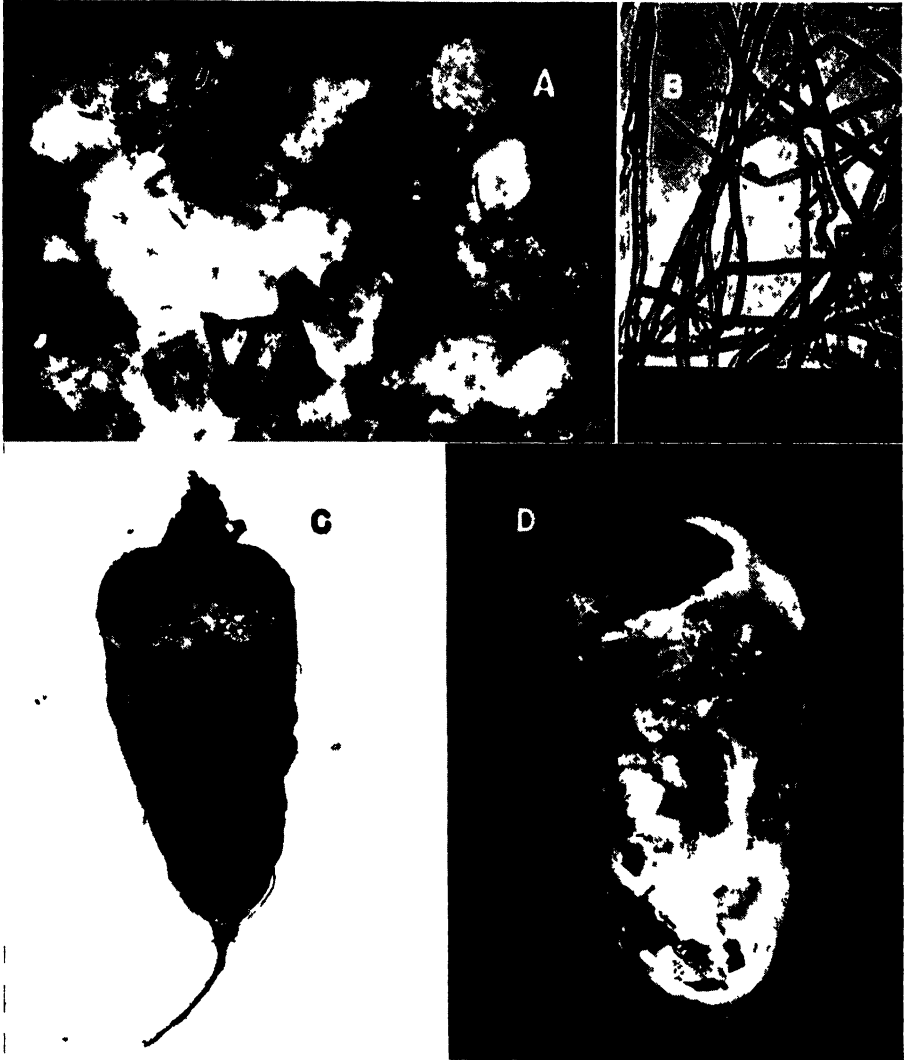
FIG. 2. Crater rot of carrots. (A) crate of carrots showing advanced stages of decay, (B) photomicrograph of hyphae of *Rhizoctonia carotae* showing clamp connections, (C) crater rot lesion invaded secondarily by *Botryotinia Fuckeliana*, (D) crater rot lesion invaded secondarily by *Sclerotinia sclerotiorum*.

name "Rhizoctonia crater rot" is suggested for this new disease.   The fungus spreads rapidly from these craters, in storages with high humidities, until entire crates are covered with the white cottony mycelium (Fig. 2, A).   The disease at this stage has an appearance similar to the rot

caused by *Sclerotinia sclerotiorum* (Lib.) Debary, which often invades these diseased tissues secondarily (Fig. 2, D). It is distinguishable from the latter by the presence of a more loose, weftlike mycelium and by the absence of sclerotia. The decayed tissues beneath the surface lesions are light brown, usually firm and not watery.

Botryotinia Fuckeliana (Debary) Whetzel (*Botrytis cinerea* Pers.) and *Sclerotinia sclerotiorum* are found commonly as secondary invaders in these lesions (Fig. 2, C). The characteristic symptoms of Rhizoctonia crater rot soon become obliterated by these more rapid-growing fungi.

Pathogenicity

Carrot roots have been repeatedly inoculated with cultures from tissue plantings of diseased roots with the production of the typical firm rot associated with the disease in storages. The same organism has been recovered from these artificially inoculated tissues. The characteristic pitting, found commonly associated with the disease in storages, has been produced in the laboratory with difficulty. Apparently an external source of nutrients is necessary for the establishment of the fungus prior to penetration. Unwashed roots with some decaying organic matter present, or washed and surface disinfested roots inoculated with a hyphal suspension containing particles of agar and incubated at high humidities (at 4°-6° C.) usually had the typical pitting on the roots. The pathogen was always recovered from lesions produced on inoculated roots.

Cultural Characters and Morphology

The mycelium of this organism in culture or on carrots is white to slightly brownish, much lighter than the common strains of *Rhizoctonia solani* Kühn. (Fig. 1, D and E). The hyphae are 2.5 to 4.0μ. in diameter, branching, with a slight constriction immediately beyond the point of branching, septate, with the septa provided with 1 to 5 clamps (Fig. 2, B).

Sclerotia are readily produced in pure culture. Their cells are hypha-like in juvenile forms and barrel-shaped in more mature sclerotia. No spores or other fructifications have been found associated with this fungus either in culture or on infected carrots in storages.

In culture, isolates of this new pathogen were different in appearance than those of the common *Rhizoctonia solani* or *R. tuliparum* (Kleb.) Whetzel and Arth. The presence of white mycelium and golden yellow to light brown sclerotia with abundant clamp connections in the hyphae separate the crater rot pathogen from these other two common pathogens in this genus present in New York.

Matsumoto (6) has made use of the fact that fusion between hyphae of different isolates in culture indicates a close relationship of the fungi in identifying diverse strains of *Hypochnus saskii* Shirai. Numerous pairings of the carrot pathogen were made by the writer with several strains of *R.*

solani and *R. tuliparum,* but no fusions were found.   Fusions were abundant in matings within strains of this new pathogen.

The presence of clamp connections in the mycelium of this fungus (Fig. 2, B) is indicative of the presence of the nuclear components necessary for sexual reproduction (1).   Strains without clamp connections on the hyphae have never been isolated.   Ramsey (7) recorded the presence of basidiospores in the lesions on the carrots which he examined, and designated the fungus as a *Hypochnus sp.*   This presumably means that the spores were echinulate and colored. However, careful microscopic examination of many hundreds of diseased carrots failed to reveal the presence of any basidia or spores which could be assigned to this fungus.   In the absence of any description of the organism by Ramsey, and the failure of the author to find any sexual spores, this organism is being placed in the form genus *Rhizoctonia* with the new name *Rhizoctonia carotae* being proposed and with the following description:

Rhizoctonia carotae sp. nov.

Hyphae hyaline to slightly brownish, 2.5 to 5.0 (mean 4.1) microns in diameter, richly branched, septate and the septa provided with 1 to 5 clamps. Mycelium white in mass on the surface of the suscept tissue or in culture.   Sclerotia golden-yellow to brownish when old, irregular, 2.5 mm. in diameter, formed loosely in the hyphae; cells of the sclerotia barrel-shaped when old, hyphalike in juvenile forms, walls brownish to yellow, 4.1 –7.1 (5.1) × 4.6–9.1 (6.2) microns, and germinating by a proliferation of one side of the cell.   Pathogenic on carrot, producing a pitting and decay of stored roots.

Found commonly in cold storages in Orleans, Monroe, and Wayne counties, New York, on carrots which have been grown on both muckland and mineral soil.

Hyphae hyalinae vel aliquantum subfuscae colore, 2.5μ – 5.0μ (medio 4.1μ) diametro, copiose ramosae, septatae, septis 1–5 clamp-connexionibus ornatis.   Mycelium album in massa in superficie tissues suscepti aut in cultura.   Sclerotia aureo-flava vel subfusca quando antiqua, irregularia, 2.5 mm. diametro, laxe in hyphis formata; cellae sclerotiorum dolliformes quando antiquae, in statibus juvenilibus similes hyphorum, parietibus subfuscis vel flavis, 4.1–7.1 (5.1) × 4.6–9.0 (6.2)μ magnitudine, per prolificem in uno cellae parieto germinantesque.   Pathogenica in Dauco Carota, foveas ruinamque in radicibus reservatis producens.

Vulgo inventa in promptuariis frigidis in comitatibus Orleans, Monroe, et Wayne Eboraci Novi in carotis qui vel in tellure stercuseo vel in metallico solo exculti sunt.

The type material and photographs have been deposited with the Department of Plant Pathology, Cornell University.   Cultures also have been sent to the American Type Culture Collection, Washington D. C. and the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

Suscepts

Inoculations of this pathogen into celery, potato, beet, parsnip, and rutabaga did not result in infection.   A white-colonied, clamp-forming organism was isolated from diseased parsnips that had been grown in

Monroe County, New York, where it was found causing a brownish rot similar to that produced by *Rhizoctonia solani*. This clamp-forming fungus appeared very similar in culture to *R. carotae;* however, inoculations into both carrots and parsnips did not produce infection. The known suscept range of *Rhizoctonia carotae*, at present, is limited to carrot roots.

Seasonal Development and Epiphytology of Rhizoctonia carotae

While little is definitely known regarding the life history of this pathogen, it is believed that the fungus may follow the same developmental pattern as some of the other species of *Rhizoctonia*. It is presumably a soil inhabitant. The disease is initiated prior to harvest time, or shortly after the roots are placed in storage. No visible symptoms are evident at harvest on carrots which later develop this disease. Carrots grown on land which was planted to other crops for several preceding years may subsequently show the disease in storage. The fact that the disease is not evident until after 1-2 months of cold storage does not necessarily indicate that infection takes place after the roots are stored, as incipient infections could take place in the field. The spotted appearance of the lesions on the roots is suggestive of penetration through areas left by dead secondary rootlets. Once lesions appear, however, the disease may develop fairly rapidly. A period of 2 to 3 weeks is usually required at 0° C. to completely destroy a root.

Storage containers may also serve as sources of primary inoculum for this disease. In one storage 19.5 per cent of over 600 isolations made from crates and baskets yielded this fungus. Direct microscopic examination has also repeatedly revealed its presence on storage containers.

The pathogen produces an abundance of mycelium over the affected areas. Adjacent roots are soon covered with the advancing mycelium and fall ready prey to its attack. The fungus grows along the wooden slats of the containers and spreads to other crates of carrots. In this way a series of secondary cycles may be initiated.

The difficulty experienced in demonstrating the several expressions of this disease in the laboratory is indicative of the rather narrow temperature and moisture requirements for infection and development of the pathogen. Observations in storages also support this contention. The greatest incidence of this disease was noted in two storages in which the humidities were maintained at near 100 per cent of saturation. The disease has never been found in common storages or in cold storages in which, by reason of construction or management, the relative humidity was held well below saturation.

The production of the pitting type of symptom in the laboratory has been produced only by wrapping inoculated carrots in wet paper toweling and placing them in a moist chamber at temperatures between 0° and 5° C.

(Fig. 1, B). The "well-method" of inoculation usually produces the typical rot associated with the pathogen.

Data presented in figure 3 indicate that on agar this organism grows over a temperature range of from –4° to 24° C., with the optimum being at 21° C. Infection has been obtained from 0° to 15° C. on carrots artificially inoculated and placed in moist chambers.
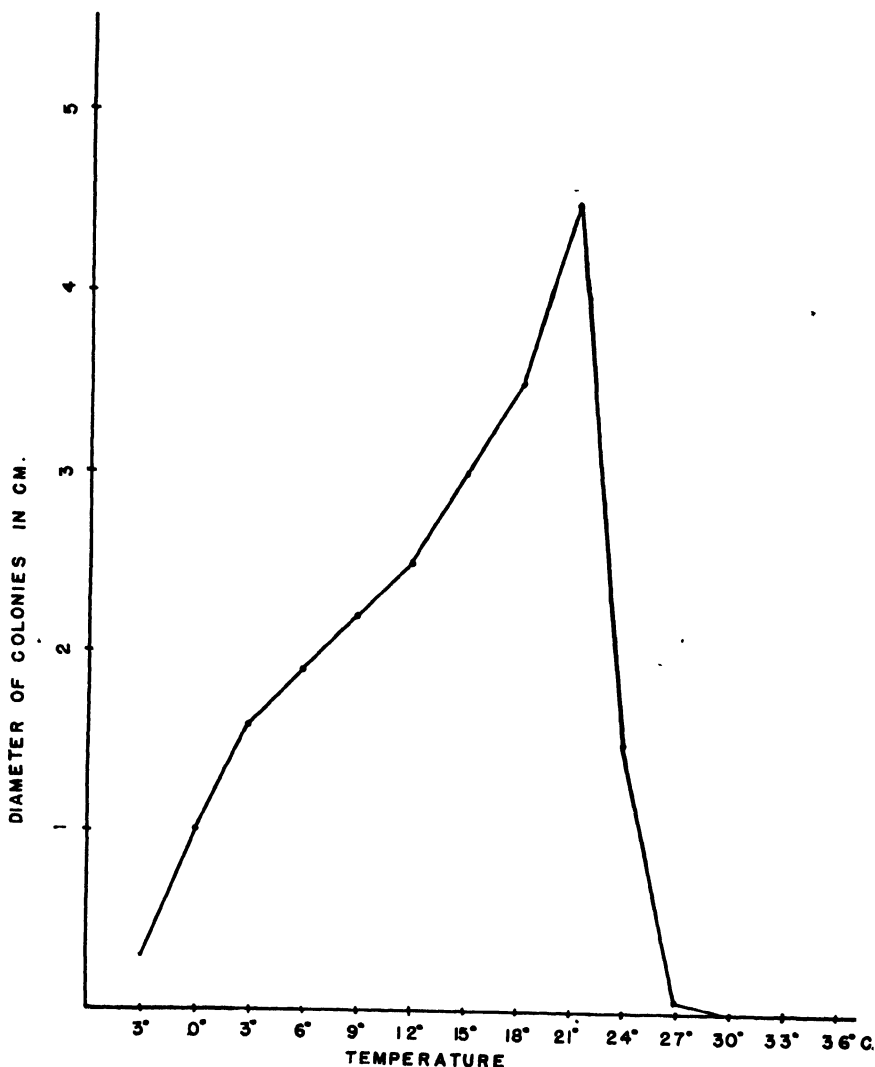
FIG. 3. Growth of *Rhizoctonia carotae* in Petri plates incubated for ten days at various temperature levels.

GLIOCLADIUM ROT

This disease occasionally caused only slight losses in stored carrots in cold storages in Monroe and Wayne Counties, New York. While losses

were never severe, the trouble was often encountered and usually con-
fused with other diseases by storage operators.

Symptomatology

The lesions on carrots appear as shallow, slightly sunken cankers on
the side of the root (Fig. 4, A). The decay is generally hard and leathery,
rarely extending into the tissues more than a few millimeters. The affected
tissues are light brown. The symptoms are very similar to those produced
by *Fusarium roseum* (Lk.) emend. Snyder and Hansen (*F. avenaceum*
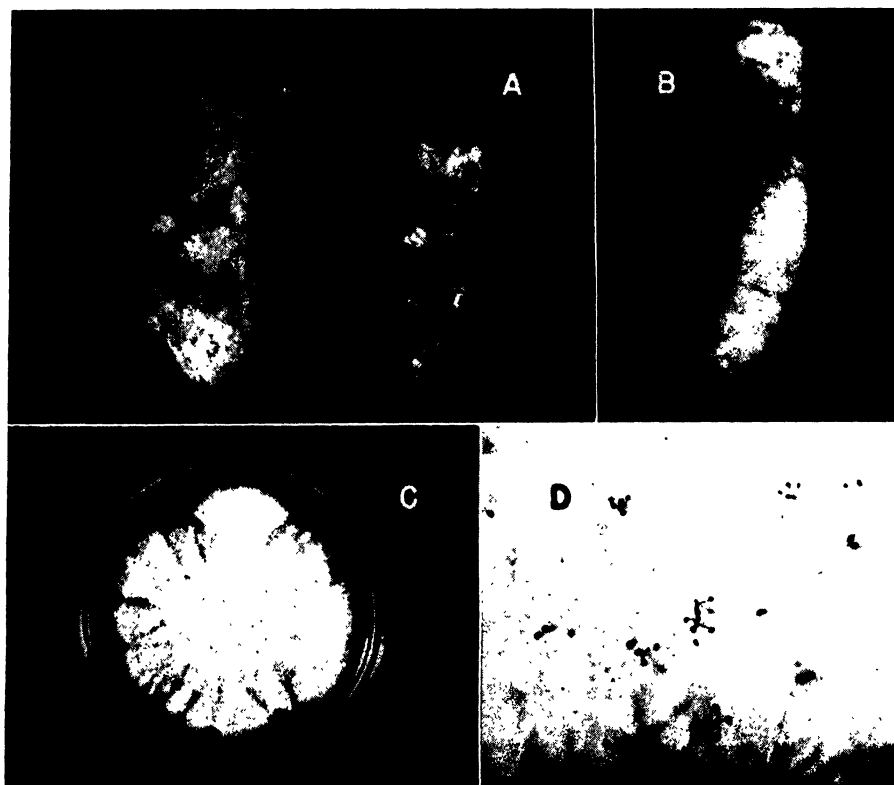
FIG. 4. Gliocladium rot of carrot. (A) shallow, hard lesions on roots from cold
storage, (B) fructifications of pathogen on carrot root incubated 10 days at 21° C.,
(C) culture of *Gliocladium aureum* sp. nov. incubated 14 days on potato-dextrose agar
at 21° C., (D) photomicrograph of Acrostalagmus type of conidial head found in young
cultures of *G. aureum*. × 60.

(Fr.) Sacc.), a fungus with which the *Gliocladium* is frequently associ-
ated. Cankers caused by *F. roseum* which have dried out are practically
indistinguishable macroscopically from those caused by the *Gliocladium*.
The decay progresses slowly, turning the tissues brown and leaving the
diseased area somewhat softer than the unaffected portion in roots inocu-
lated by placing small bits of mycelium in holes bored in the carrots and
incubated in moist chambers at 6° C. The affected area frequently is cov-

ered with a thick mat of mycelium (Fig. 4, B). The lesions are readily invaded by *Botryotinia Fuckeliana, Mucor hiemalis* Wehmer, or *F. roseum,* which quickly mask the characteristic lesions of this disease.

Etiology

Isolations from characteristic lesions have consistently yielded a fungus, which, by the characters of the conidia and conidiophores falls within the generic limits of the genus *Gliocladium.* Pathogenicity of this organism has been repeatedly demonstrated by artifical inoculations into carrots.

A careful study of the isolate from carrots has shown it to be very closely related to *Gliocladium roseum* (Lk.) Bainier. *G. roseum* was first described by Link (5) on dead stems of *Solanum* sp. under the name *Penicillium roseum* Lk. Lindau (4) listed this organism on dead stems of potato and *Hibiscus* sp., on decayed carrot roots, dead *Boletus* spp. and sclerotia in Germany, Belgium, Holland, and Ceylon. Saccardo (8) noted the fungus also as occurring on carrots in the Netherlands. Ravenel (Fungi Americani No. 571) collected this fungus (also under the name *Penicillium roseum*) from South Carolina on leaves of *Buxus sempervirens.* It has been noted by other authors as a laboratory contaminant and as occurring in the soil. As far as the writer has been able to determine *G. roseum* has never been found as a pathogen of living plants.

Sketches of an organism made by Shapovalov (9) under the name of *Clonostachys araucaria* DC. var. *rosea* Preuss. resemble very closely the pathogen found on carrots. The figures given by Shapovalov, however, are not *Clonostachys araucaria* in the sense of Lindau (4, page 345 and figure on page 347) since the sterigmata are not shown to branch in a verticillate manner, and the conidia are borne catenulately on the phialids. The conidia are not long-spicate nor are the spikes in a dense cluster as described by Saccardo (8, p. 165 in vol. 4) or Clements and Shear (2) and pictured by Lindau (4). The presence of a true penicillus is pictured by Shapovalov, some of the conidial clusters being united into a mucilaginous head typical of species of *Gliocladium.* Also, the Acrostalagmus type of head (10, figure 89; 3, page 442, figure 229, c) frequently found in cultures of *Gliocladium* spp. is also shown in Shapovalov's figure 2.

The distinct apiculum described by Shapovalov on the conidia of the organism that he found attacking potato has also been noted on the conidia of the carrot pathogen. The presence of the Acrostalagmus type of conidial head in conjunction with the true *Gliocladium* fructifications present in cultures of both the potato and carrot pathogens indicates that these organisms are probably the same species (Fig. 5).

Gliocladium roseum (*Penicillium roseum*) collected by Ravenel (Fungi Americani No. 571) and also by Meschutt (C.U.P.P. No. 6790)[2] on dead leaves of *Buxus sempervirens* has been examined and found to be different from the carrot pathogen.

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[2] A specimen, No. 6790, determined by J. B. Ellis, filed in the herbarium of the Department of Plant Pathology, Cornell University, Ithaca, New York.

The general morphological characters of the pathogen found on carrot
place it close to *Gliocladium roseum*. However, the slightly curved shape
of the conidia combined with active pathogenicity, makes it seem advisable
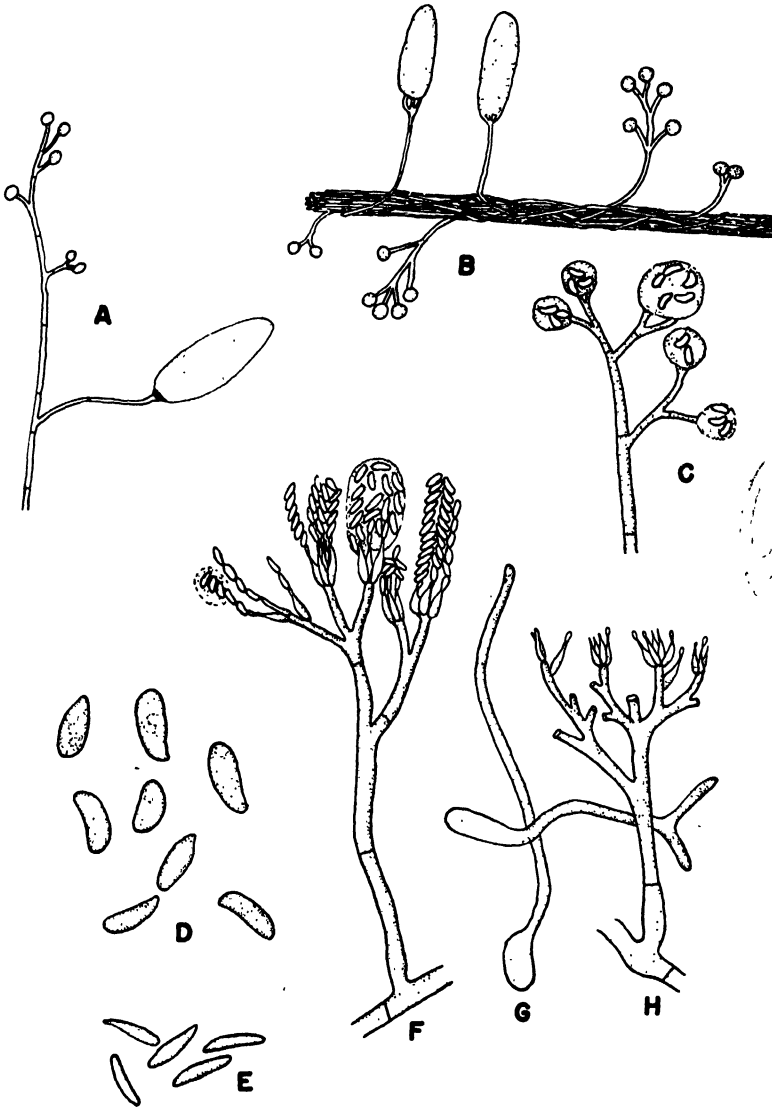
FIG. 5. *Gliocladium aureum* sp. nov., camera lucida drawings. (A) single hypha,
bearing both Acrostalagmus and true Gliocladium types of fruiting structures, (B)
hyphae bundled into strands (found commonly on agar cultures), (C) close-up of
Acrostalagmus type of fruiting structures, (D) conidium of *G. aureum*, (E) conidia of
G. roseum (*P. roseum*, Ravenel, Fungi Americani No. 571), (F and H) details of the
penicillus of *G. aureum*, (G) germinating conidia of *G. aureum*, (A, B, approx. × 160;
C, F, H, approx. × 480; D, E, G, approx. × 1450).

to distinguish this fungus from the common saprogen by designating it
as a new species. The name *Gliocladium aureum* is proposed with the fol-
lowing description:

Gliocladium aureum sp. nov.

Colonies on potato-dextrose agar or Czapek's medium pure white at first, later pale yellow, occasionally light salmon to pink in the fruiting areas on Czapek's medium; mycelium loose-floccose, composed of simple hyphae or hyphae bundled in ropes; sclerotia not observed; conidiophores usually perpendicular to the medium, 40–120μ; penicillia up to 130μ in length, once or twice irregularly alternately branched; phialids 10–15 × 2–3μ. bearing conidia which may be aggregated in gelatinous masses or balls (Acrostalagmus type) or agglutinated in typical columns (resembling the Clonostachys type); conidia hyaline, elliptical, apiculate, smooth, 3.3 × 5.9μ. (extremes 2.6–3.6 × 5.1–6.7μ). A slight to marked yellowing of the medium is present in cultures on potato-dextrose agar.

Habitat: Pathogenic on stored carrot roots in New York.

Coloniae, in Solano tuberoso dextroso agari aut in Czapekis medio, primum purae albae, recentior pallidae flavae, pallidae salminae vel rosaceae in locis frugiferis in Czapekis medio; Mycelium laxe floccidum, compositum ex hyphis simplicibus aut ex hyphis in funes coactis; sclerotia non observata; conidiophoria plerumque ad medium perpendicularia, 40–120μ; penicillia ad 130μ longitudine, semel bisve verticillato aut irregulariter alterne ramosa; phialids 10–15 × 2.0–3.0μ ferentes conidia aut ad massas gelatinosas globulosve aggregata (Acrostalagmus typus) aut ad columnas typicas agglutinata (similes Clonostachys typi); conidia hyalina, elliptica, apiculata, laevia, 3.3 × 5.9μ (extremis 2.6–3.6 × 5.1–6.7μ) Levis vel notata medii flavido in culturis in Solano tuberoso dextrose agari adest.

Pathogenicum in Dauci Carota radicibus reservatis in Eboraco Novo.

The type material and photographs have been deposited with the Department of Plant Pathology, Cornell University. Cultures have also been sent to the American Type Culture Collection, Washington, D. C., and the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

No data are available on the exact life history of this fungus. However, its similarity to species of *Penicillium*, both in distribution and cultural requirements would probably indicate a similar seasonal development. This would mean a succession of cycles on roots throughout the year whenever the suscept is present and proper environmental conditions exist.

Studies on the growth of this fungus at various temperatures indicate an optimum temperature between 21° and 24° C., a minimum below –3° C., and a maximum between 33° and 36° C. Infection has been obtained regularly on inoculated roots incubated in moist chambers at 6° and at 21° C.

DISCUSSION

The two diseases described are a part of a complex of four diseases causing pitting of stored carrot roots in New York. The other two diseases are caused by *Fusarium roseum* (Lk.) emend. Snyder and Hansen and by several species of *Typhula*. The *Typhula* occurs infrequently and the disease is characterized by the absence of hyphae in the lesions and by the typical brownish to blackish sclerotia on the affected areas. Frequently it is impossible to separate the diseases caused by *Rhizoctonia carotae, Gliocladium aureum,* and *Fusarium roseum* with certainty without microscopic examination. All three organisms produce pits varying in size from 2 to

3 mm. to several centimeters in diameter. These pits are usually lined with white hyphae. Typical expressions of these diseases permit separation as follows:

Rhizoctonia crater rot (*R. carotae*); sunken lesions lined with white mycelium, clamp connections present in the hyphae; affected tissues beneath the surface of the lesions light brown; in advanced stages hyphae readily spread to adjacent roots.

Fusarium rot (*F. roseum*); sunken lesions lined with white to yellow-ish tan or reddish mycelium, usually having a granular appearance due to the production of spores by the pathogen; hyphae lacking clamp connections, and rarely spreading to adjacent roots. Affected tissues are only slightly discolored, dry, and punky.

Gliocladium rot (*G. aureum*); lesions only slightly sunken, sparsely lined with light yellowish to pinkish mycelium; affected tissues light tan, very hard and leathery.

Gliocladium and Typhula rots are of less frequent occurrence and on the basis of present knowledge would not be expected to cause any serious losses in stored carrots.

Fusarium rot may become severe at temperatures of 15° to 20° C.; although its presence has been detected in carrots stored at temperatures between 6° and 35° C. While these temperatures occasionally exist in common storages this disease seldom causes any serious damage because of the low humidity in these storages.

The presence of Rhizoctonia crater rot in storage is dependent upon either field contamination or infection, or contamination from storage containers. Uninfected or uncontaminated roots placed in clean containers will not develop this disease.

Rhizoctonia carotae is the only pathogen in this group of pit producing fungi which causes any severe damage in cold storage. All experiments on control of this disease have given inconclusive results. Washing roots prior to storage has greatly increased the amount of soft rot (*Erwinia carotovora* (Jones) Holland) developing during the storage period with little effect on the incidence of Rhizoctonia crater rot. Dipping the roots in fungicides has not consistently controlled this disease.

Carrots stored in clean or disinfected crates and placed in storage rooms maintained at 0° C. and at a relative humidity of 90 - 95 per cent of saturation have shown a lower incidence of crater rot. This is the only control measure which can be recommended.

SUMMARY

Two diseases of carrots found in New York storages are described as new.

Rhizoctonia crater rot (*Rhizoctonia carotae* n. sp.) has been found caus-

ing severe losses in certain cold storages where the relative humidities have been high. This disease is characterized by sunken lesions lined with a white flocculent mycelium. These lesions may occur anywhere on the root. The affected tissues are light brown, usually firm and not watery. The hyphae in the lesions and in culture are hyaline to slightly brownish, 2.5 to 5.0μ (mean 4.1μ) in diameter, richly branched, 1–5 clamps at the septa. Sclerotia golden yellow to brownish, formed loosely on the hyphae, cells of the mature sclerotia barrel-shaped, and germinating by a proliferation of one side or the ends.

Gliocladium rot (*Gliocladium aureum* n. sp.) has been found in both cold and common storages in New York. This disease is characterized by shallow, slightly sunken lesions on the sides of the roots, which lesions are sparsely lined with light yellowish to pinkish mycelium; the affected tissues are very hard and leathery and light tan.

The only control believed recommendable is to store carrots at relative humidities below 95 per cent in new or disinfected containers.

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IMMUNITY OF CANADIAN BLACK CURRANT SELECTIONS FROM BLISTER RUST

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The standard horticultural varieties of the European black currant, *Ribes nigrum* L., as commonly grown in this country are regarded as a definite menace to five-needle pines here because of their extreme susceptibility to white-pine blister rust, *Cronartium ribicola* A. Fisch. An investigation of these as well as a very large number of *Ribes* species for rust susceptibility, as listed by Spaulding (7), who reported his own work and that of his associates (7, pp. 17-24) a quarter of a century ago, did not reveal any black currant species resistant to blister rust. The early investigations, however, were lacking in studies of Asiatic black currant species from the probable original habitat of *C. ribicola* (3, p. 739) where rust-resistant ribes most likely would be found.

As the result of subsequent investigations of ribes susceptibility, the writer (2) found that varieties of *Ribes nigrum,* on the other hand, showed a marked resistance to piñon blister rust, *Cronartium occidentale* Hedgc., Beth., and Hunt. Accordingly, it was reasonable to expect that in the black currant group (1, pp. 29-38), as the writer proved subsequently for the red currant group (4), species and varieties would be found highly resistant to, or immune from *C. ribicola.* Experiments described in this paper are concerned with an Asiatic member of the black currant group.

CANADIAN EXPERIMENTS TO DISCOVER RUST-RESISTANT BLACK CURRANTS

In recent years a search was undertaken at the Central Experimental Farm, Ottawa, by Hunter and Davis (6) for suitable black currants for hybridization purposes in breeding for rust resistance. One black currant in particular from Siberia was used. It was called tentatively *Ribes nigrum,* No. 19/11. In 1939 Professor Alfred Rehder, Arnold Arboretum, Harvard University, identified it as *R. ussuriense* Jancz., from eastern Manchuria, the twin species of *R. nigrum,* which, to quote Berger (1, p. 32) "differs [from *nigrum*] in many ways, and of quite a different odor." According to a communication received from Mr. D. S. Blair, September 23, 1941, concerning field tests carried on during 1935-40 at the Central Experimental Farm, the Asiatic black currant species, not tested for rust susceptibility in the United States, showed no signs of rust infection although it was cultivated in close association with *nigrum* varieties that became heavily infected with rust. *R. ussuriense,* according to Hunter and Davis (6), is apparently homozygous, rust-resistant.

Among the most desirable hybrids obtained were those that resulted from crossing pistillate *Ribes ussuriense* with staminate *R. nigrum* var. Kerry

(6). The Kerry variety is of Canadian origin and is a seedling of Black Naples. Both of these standard *nigrum* varieties are rust-susceptible. Two selections from these crosses, namely No. 0-381 and 0-393 reported in this paper, represent separate clones respectively, each the vegetative progeny of a single seedling. Hunter and Davis (6) state that they show a marked resemblance in fruit characteristics to their cultivated parent, particularly with respect to vitamin C content and desirability for jam production. In the case of the Viking red currant (3), resistance to *Cronartium* is dominant to susceptibility; in the Canadian black currant selections (6), No. 0-381 and 0-393, the same inheritance appears to hold true.

INOCULATION RESULTS WITH CANADIAN BLACK CURRANTS

In the autumn of 1945, through the courtesy of Mr. M. B. Davis, Dominion Horticulturist, and Mr. A. W. S. Hunter, three plants each of the Canadian black currant clones No. 0-381 and 0-393 were received at New Haven for pathological testing[2]. The plants were grown first in pots in an unheated greenhouse. On April 11, 1946, the six plants were inoculated in the greenhouse with freshly collected aeciospores[3] from Connecticut, according to methods described previously (3, p. 741). Susceptible ribes "checks" were used to establish the viability of the inoculum and the suitability of the environmental conditions for infection. The checks included an undetermined variety of *Ribes nigrum*; the native black currant, *R. americanum* Mill.; and selections of a black currant propagated at the C. A. Hansen .Nursery, Brookings, South Dakota, and reported as having been originally introduced from Siberia by Dr. N. E. Hansen in 1897 (5, p. 45; 8, p. 28). Through the courtesy of C. A. Hansen, the Hansen black currant selections were obtained in June, 1945. All of the checks became infected, the *nigrum* variety so heavily as to cause defoliation, whereas the other two black currant checks became only moderately infected. When inoculated, the plants of the two Canadian black currant clones had well developed leaves, which were in prime condition for the single test made that year. No signs of rust infection developed on them.

During the late summer of 1946 two plants of each clone were transplanted outdoors to a ribes plantation for testing under field conditions the following year. In 1947 the two plants retained in the greenhouse were

inoculated on April 30 with fresh aeciospores of *Cronartium ribicola* collected in Virginia and later, on May 20, with the same type of inoculum collected in Connecticut. The susceptible ribes used as checks consisted of skunk currant, *R. glandulosum* Grauer, and a variety of *Ribes nigrum*. The two clones transplanted to the field were inoculated under conditions of humidity, temperature, and viability of inoculum that were highly favorable to infection. A test with inoculum from Connecticut was made on May 22 and a second test with inoculum from Maine on June 14. The susceptible field checks consisted of the same *nigrum* variety used in the greehouse and the Hansen black currant. Very heavy infection of *C. ribicola* occurred on the greenhouse checks; on the field checks infection was moderate on *nigrum* whereas that on the Hansen black currant was slight. There were no signs of rust infection on the Canadian black currants growing either in or outside the greenhouse[4]. Under the conditions of the tests the Canadian black currants appear to be truly immune from blister rust in the nice sense of that term, *i.e.*, completely free or exempt from rust. In this respect the black currant clones differ from the commercial Viking red currant, for at certain stages of leaf development in this particular ribes an invasion of germ tubes of *C. ribicola* occurs but the infectious agent is totally unable to establish itself and subsequently to produce its spores (4, p. 343).

RESULTS OF NATURAL EXPOSURE TO RUST AND MILDEW

During the tests of 1946 and 1947 uredospores of blister rust were present in the greenhouse and in the field, as indicated by natural infection of *Cronartium ribicola* throughout the season on leaves of susceptible ribes not inoculated artificially. Infection of the Canadian black currants was not observed to occur from inoculum of this type.

In 1946, after results of the rust-inoculation experiments on the Canadian clones in the greenhouse had been obtained, a natural infection of powdery mildew, *Sphaerotheca mors-uvae* (Schw.) Berk. and Curt., occurred on the new growth. Conditions in the unheated greenhouse were so favorable for mildewing that even the *nigrum* check, not ordinarily attacked, became affected. In the late spring of 1947 mildew again occurred in the greenhouse but it was not nearly so severe as in the previous year. It did not occur, however, on the Canadian clones in the field. These observations indicate, as Hunter and Davis (6) had previously observed, that the basis of resistance to blister rust in the two black currant clones is not operative with respect to mildew.

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[4] In a letter dated October 4, 1947, Professor A. J. Riker informed the writer that the Canadian black currant clones sent him for testing have been growing in pots in a greenhouse along with other black currants. So far the former have not shown any blister rust whereas the latter have been severely rusted. Accordingly the Canadian clones are recorded to date as immune from rust inoculum collected in Connecticut, Maine, Virginia, and Wisconsin.

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EFFECT OF COPPER INJURY ON CONCORD GRAPES[1]

R. F. SUIT[2]

INTRODUCTION

Ever since Bordeaux mixture was discovered and it was found that this fungicide would control downy mildew (*Plasmopara viticola* (Berk. and Curt.) Berl. and De Toni) on grapes (7), it has been generally considered that grapes were not particularly subject to copper spray injury. How-ever, in 1941 a preliminary report (2) indicated that Concord grapes were subject to injury from sprays containing neutral copper fungicides at a copper content equal to that in a 4-4-100 Bordeaux mixture. The injury was characterized by reduced vine growth, smaller, yellowish-green leaves and reduced yield. Subsequently it was reported (3, 4) that the yield obtained the year following the spray application was reduced more, even though a copper fungicide was not applied. The second year following the injury, the yield from the injured vines had increased so that it was equal to that from vines which had not been injured (4).

Because of this apparently severe copper injury to Concord grapes, it was advisable to determine the exact effect of the injury over a period of years, the concentration of copper that would not produce injury and a method of alleviating the injury. Although Bordeaux mixture did not visibly injure the vines it could not be used with certain insecticides for insect control on grapes because of its alkaline reaction. Therefore, it was necessary that a neutral fungicide be used under some conditions.

MATERIALS AND METHODS

Through the cooperation of the Pomology Division at Geneva, New York, a block of Concord grapes, 80 years of age, was made available at the Vineyard Laboratory, Fredonia, New York for the investigation of copper injury. The block was of sufficient size so that 16 spray treatments could be used with duplicate plots of 30 vines each. There were no diseases present in this vineyard, but the grape berry moth (*Polychrosis viteana* Clem.) was present which made some modification of the spray schedule necessary (6).

The complete spray schedule was as follows:

Application   I—When 3 or 4 leaves were present on the new shoots.

Application   II—Before bloom.

Application III—After bloom.
Application IV—Ten to fourteen days after application III.
Application V—When second brood grape berry moth began to appear.
Application VI—Ten to fourteen days after application V.

Thus a complete schedule including the control of the grape berry moth required 6 applications. Normally a maximum of 5 applications may be used for black rot (*Guignardia bidwellii* (Ell.) Viola and Ravaz.) control with the fifth application made 4 weeks after bloom.

Tribasic formulae
  A. Tribasic copper sulfate 2–100
      Rosin fish oil soap 1½–100
  B. Tribasic copper sulfate 2–100
      Black Leaf 155 (14 per cent) 3–100
      Rosin fish oil soap ¾–100
  C. Tribasic copper sulfate 2–100
      Hydrated lime 4–100
      Rosin fish oil soap 1½–100
  D. Tribasic copper sulfate 2–100
      Lead arsenate 3–100
      Hydrated lime 4–100
      Rosin fish oil soap 2–100
      Kerosene—1 pint–100
  E. Tribasic copper sulfate 1–100
      Black Leaf 155 (14 per cent) 3–100
      Rosin fish oil soap ¾–100
YCO formulae
  A. Yellow cuprocide 1–100
      Rosin fish oil soap 1½–100
  B. Yellow cuprocide 1–100
      S.E.C. oil[1] 1 pint–100
  C. Yellow cuprocide 1–100
      Black Leaf 155 (14 per cent) 3–100
      Rosin fish oil soap ¾–100
  D. Yellow cuprocide 1–100
      Black Leaf 155 (14 per cent) 3–100
      S.E.C. oil 1 pint–100
  E. Yellow cuprocide 1–100
      Hydrated lime 4–100
      Rosin fish oil soap 1½–100
  F. Yellow cuprocide 1–100
      Hydrated lime 4–100
      S.E.C. oil 1 pint–100
  G. Yellow cuprocide 1–100
      Lead arsenate 3–100
      Hydrated lime 4–100
      Rosin fish oil soap 2–100
      Kerosene 1 pint–100

YCO formulae
  H. Yellow cuprocide 1–100
      Lead arsenate 3–100
      Hydrated lime 4–100
      S.E.C. oil 1 pint–100
  I. Yellow cuprocide 10 oz.–100
      Black Leaf 155 (14 per cent) 3–100
      Rosin fish oil soap ¾–100
Lead and lime formula
      Lead arsenate 3–100
      Hydrated lime 4–100
      Rosin fish oil soap 1½–100
Black Leaf formula
      Black Leaf 155 (14 per cent) 3–100
      Rosin fish oil soap ¾–100
Bordo formulae
  A. Bordeaux mixture 2–2–100
      Rosin fish oil soap 1½–100
  B. Bordeaux mixture 2–2–100
      Calcium arsenate 3–100
      Rosin fish oil soap 2–100
      Kerosene 1 pint–100
  C. Bordeaux mixture 2–2–100
      S.E.C. oil 1 pint–100
  D. Bordeaux mixture 2–2–100
      Calcium arsenate 3–100
      S.E.C. oil 1 pint–100
  E. Bordeaux mixture 4–4–100
      Rosin fish oil soap 1½–100
  F. Bordeaux mixture 4–4–100
      Calcium arsenate 3–100
      Rosin fish oil soap 2–100
      Kerosene 1 pint–100
  G. Bordeaux mixture 8–8–100
      Rosin fish oil soap 1½–100
  H. Bordeaux mixture 8–8–100
      Calcium arsenate 3–100
      Rosin fish oil soap 2–100
      Kerosene 1 pint–100

[1] S.E.C. oil is self-emulsifying cottonseed oil supplied by Rohm and Haas Co.

Because of the variation in the insecticides used in the spray mixtures for the different applications (6) the number of spray mixture formulae necessary for the experiment was materially increased. In the 24 formulae listed, all materials are given as pounds per 100 gallons of water unless otherwise stated. In some of the spray mixtures it will be noted that 1 pint of kerosene is added per 100 gallons. This is added to reduce the foaming resulting from the use of excess rosin fish oil soap which was neces-

sary to give the mixture a suitable physical property so that it would wet
and cover the grape berries with a satisfactory spray deposit (6).

The treatments were arranged to allow for plots sprayed with neutral
copper fungicides alone, with different spreader stickers and with lime,
while the Bordeaux mixture plots were sprayed with different concentra-
tions and with different numbers of applications. The spray mixtures
applied to the plots for each of the 16 treatments and for each of the 6
applications are given in table 1.

TABLE 1.—*Spray mixtures used for the various treatments*

Treatment No.	Application					
	I	II	III	IV	V	VI
1	Tribasic–A	Tribasic–A	Tribasic–B	Tribasic–B	Tribasic–B	Tribasic–B
2	YCO-A	YCO-A	YCO-C	YCO-C	YCO-C	YCO-C
3	YCO-B	YCO-B	YCO-D	YCO-D	YCO-D	YCO-D
4	None	None	Lead & Lime	Lead & Lime	Black Leaf	Black leaf
5	Bordo-A	Bordo-A	Bordo-B	Bordo-B	Tribasic–E	Tribasic–E
6	Bordo-C	Bordo-C	Bordo-D	Bordo-D	Tribasic–E	Tribasic–E
7	Bordo-E	Bordo-E	Bordo-F	Bordo-F	Tribasic–E	Tribasic–E
8	Bordo-G	Bordo-G	Bordo-H	Bordo-H	Tribasic–E	Tribasic–E
9	None	Bordo-G	Bordo-H	Bordo-H	Tribasic–E	Tribasic–E
10	None	None	Bordo-H	Bordo-H	Tribasic–E	Tribasic–E
11	None	None	Black Leaf	Black Leaf	Black Leaf	Black leaf
12	Tribasic–C	Tribasic–C	Tribasic–D	Tribasic–D	Tribasic–E	Tribasic–E
13	YCO-E	YCO-E	YCO-G	YCO-G	YCO-I	YCO-I
14	YCO-F	YCO-F	YCO-H	YCO-H	YCO-I	YCO-I
15	Bordo-G	Bordo-G	Tribasic–B	Tribasic–B	Tribasic–B	Tribasic–B
16	Bordo-A	Bordo-A	Tribasic–B	Tribasic–B	Tribasic–B	Tribasic–B

The spray mixture was applied at 350-lb. pressure with a trailing hose
to which was attached a two-nozzle boom that had No. 4 discs in the noz-
zles. The amount of spray mixture used varied with the amount of growth
on the vines. For application III the rate was about 225 gal. per acre,
which is the amount recommended for satisfactory grape berry moth con-
trol and which would also give satisfactory disease control. In practically all
cases the spraying of the plots for each application was completed in one
day.

The experiment was carried on from 1941 to 1944 inclusive and the
exact date on which each application was made during those years is given
in table 2. In each year the crop of fruit was harvested and yield records
obtained during the latter part of September.

RESULTS

The first indication of copper injury was a reduction in the amount
of cane growth coupled with the yellowish-green color and reduced size of
the leaves. Those rows in the vineyard which had copper injury could be
noted from some distance because of the lighter green color. In 1941 and
1944 the early appearance of noticeable injury did not occur. However,
in 1942, the injury was present on June 1, the date on which application

TABLE 2.—*Dates of spray applications*

Year	Application					
	I	II	III	IV	V	VI
1941	May 19	June 2	June 23	July 11	August 4	August 18
1942	May 19	June 1	June 23	July 7	July 29	August 7
1943	June 4	June 15	July 7	July 15	July 28	August 13
1944	May 25	June 6	June 26	July 11	July 31	August 11

II was made and prior to the blooming period. The injury was first noticed on July 7 in 1943, just after the blooming period and the date on which application III was made. In 1942 the injury appeared 13 days after the first application, while in 1943 it appeared 33 days after the first application. The vines had been severely injured by the cold during the winter of 1942-43 and were retarded in their growth.

A study of the effect of spray treatment on the amount of cane growth was started in 1942. Exceptionally heavy winds occurred during the latter part of May and broke off many of the canes that were being measured so that it was not possible to complete the experiment. In 1943, measurements were again made but, because of the injury from the cold during the previous winter, complete records could not be made. A summary of the measurements on some of the plots is given in table 3. The neutral coppers (treatments 1 and 2) and the Bordeaux mixture 8-8-100 (treatment 8) caused a greater reduction in cane growth than the other treatments and the growth was about half as much as that which occurred when copper was not applied (treatment 4). A complete series of measurements was made in 1944. As shown in table 4 there was no significant difference between treatments 1, 2, 5, 7, and 8 which comprised the neutral coppers and the three concentrations of Bordeaux mixture. Significantly more growth occurred in treatments 4, 11, 12, and 13 which were the two check plots and

TABLE 3.—*Effect of certain spray treatments on length of cane growth in 1943.*

Treatment No.	Principal fungicide	Inches of growth on				
		June 4	June 15	July 7	July 15	July 28
1	Tribasic	14.8 a	29.6	48.6	54.3	60.6
2	YCO	15.1	27.2	49.4	60.2	71.0
4	Lead and lime	16.3	38.9	89.5	117.8	144.4
7	Bordo 4-4-100	19.0	44.3	71.4	91.4	110.5
8	Bordo 8-8-100	14.6	28.8	52.0	63.2	75.6
13	YCO–Lime	18.8	37.2	69.8	84.8	98.6

a Average of measurement of from 5 to 10 canes in each plot.

the two neutral coppers plus lime. These differences occurred even though there was no apparent visible symptom of injury during the early part of the season. These results on cane growth indicate that the neutral coppers at a copper concentration equal to Bordeaux mixture 4–4–100 will cause severe reduction, that Bordeaux mixture usually causes some reduction, and that the neutral coppers with lime will cause the least reduction in growth.

Since the copper fungicides cause a reduction in the cane growth it is probable that a reduction in yield should also occur. The results from the yield records taken from 1941 to 1944 inclusive are given in table 5.

TABLE 4.—*Effect of certain spray treatments on length of cane growth in 1944.*

Treatment No.	Principal fungicide	Inches of growth [a] on						
		May 26	June 5	June 27	July 12	Aug. 1	Aug. 10	Oct. 4
1	Tribasic	13.5	28.8	42.7	52.3	60.8	64.6	72.5
2	YCO	13.5	28.6	43.1	55.7	65.2	72.7	82.0
4	Lead and lime	15.7	35.1	59.5	81.3	99.2	109.6	125.2
5	Bordo 2-2-100	14.2	29.4	46.9	55.8	65.7	69.4	75.9
7	Bordo 4-4-100	14.3	30.1	46.5	60.0	69.2	73.3	79.1
8	Bordo 8-8-100	14.5	29.1	46.9	61.8	72.1	78.9	86.7
11	Black Leaf	13.9	32.2	57.3	76.7	95.4	107.5	122.5
12	Tribasic– lime	15.6	31.6	53.2	75.1	91.3	99.1	112.3
13	YCO–lime	14.4	30.5	50.0	67.8	85.5	94.8	109.7

Least difference needed for significance—odds 19:1   18.28
                                         odds 99:1   24.11

[a] Average of measurements of 24 canes in each spray treatment.

There was no significant difference in the yield from any of the treatments in 1941. There was a significant reduction in the yield from treatments 1, 2, 3, 5, 6, 7, 8, 12, 13, 14, 15, and 16 in 1942. Although the yield in 1943 was reduced by the winter injury, there was a significant reduction in the yield from treatments 1, 2, 3, 7, 15, and 16. The 1944 results did not show as great a variation among the treatments and are somewhat of the same order as those obtained in 1941, although treatments 1, 13, and 16 gave a significant reduction in yield. In each of the four years, treatment 4 (lead and lime check) which did not contain copper gave the highest yields. Apparently the Black Leaf 155 (treatment 11) was somewhat injurious since the yields were slightly less than those from the lead and lime (treatment 4) although the difference was not significant. Considering the average

of the yields for the 4-year period, the data indicate that the neutral
coppers (treatments 1, 2, 3) caused the greatest reduction in yield. Treat-
ments 15 and 16 in which Bordeaux mixture was used for applications I
and II and tribasic copper sulfate for applications III and IV, were
equally injurious. Four of the Bordeaux treatments (No. 5, 6, 7, and 8)
and one (No. 13) of the neutral copper resulted in a significant reduction

TABLE 5.—*Effect of spray treatments on yield*

Treatment No.	Principal fungicide	Yields				4-year average
		1941	1942	1943	1944	
1	Tribasic	9.8	3.3	2.9	5.3	5.3
2	YCO	9.4	3.1	2.4	7.6	5.6
3	YCO	10.6	5.8	1.9	8.3	6.7
4	Lead and lime	11.3	14.8	6.9	10.0	10.7
5	Bordo 2-2-100	9.8	9.1	4.6	9.8	8.3
6	Bordo 2-2--100	9.3	10.5	4.7	8.8	8.4
7	Bordo 4-4-100	9.4	8.3	3.1	8.1	7.2
8	Bordo 8-8-100	9.1	9.3	4.3	8.4	7.8
9	Bordo 8-8-100	9.3	12.1	5.1	8.1	8.7
10	Bordo 8-8-100	9.2	12.7	4.8	8.0	8.7
11	Black Leaf	10.1	13.3	5.3	9.0	9.4
12	Tribasic-lime	10.6	10.6	4.9	8.5	8.7
13	YCO-Lime	9.4	10.5	4.6	6.7	7.8
14	YCO-Lime	10.1	10.6	6.1	7.7	8.6
15	Bordo-Tribasic	8.1	7.8	2.5	7.1	6.4
16	Bordo-Tribasic	9.1	7.7	2.1	6.9	6.5
Least difference needed for significance	Odds 19:1	3.06	3.06	3.06	3.06	2.17
	Odds 99:1	4.23	4.23	4.23	4.23	2.90

ᵃ Yield—average yield in pounds of fruit per vine from duplicate plots of 30
vines each.

in yield compared to that from the lead and lime (No. 4) check. The other
treatments did not differ significantly in yield from that of the treatment 4.

While recording the yield data in 1942 it was observed that the bunches
of grapes harvested from the vines in the plots which had copper injury
appeared to be small. Therefore, when the yields were taken in 1943 and
1944, weights of 100 bunches from each plot were recorded (table 6.) The
data show that those treatments which caused a significant reduction in
yield (table 5) also caused a significant reduction in the size of the bunches.

The data presented show that copper injury on grapes causes a reduction in cane growth, yield, and size of bunches. However, this difference was not so apparent in some years as in others. It has been reported (1) that wet weather gives favorable atmospheric conditions for copper injury on apples. Copper injury was first observed on grapes in 1940 (2, 4) and caused a reduction in yield. In the experiments herein reported, the greatest reduction in yield occurred in 1942 and 1943. An examination of the weather records for May, June, July and August from 1940 to 1944,

TABLE 6.—*Effect of spray treatment on size of bunches*

Treatment No.	Principal fungicide	Weight (in pounds) of 100 bunches		
		1943	1944	2-year average
1	Tribasic	16.2	13 5	14.8
2	YCO	16.6	14.9	15.8
3	YCO	14.0	14.1	14.1
4	Lead and lime	20.0	18.1	19.0
5	Bordo 2–2–100	18.0	16.6	1ᵔ.3
6	Bordo 2–2–100	17.8	15.7	16.8
7	Bordo 4–4–100	18.3	16.1	17.2
8	Bordo 8–8–100	18.2	15.3	16.8
9	Bordo 8–8–100	17.5	17.3	17.4
10	Bordo 8–8–100	18.2	17.6	17.9
11	Black Leaf	20.0	17.6	18.8
12	Tribasic–lime	20.5	16.8	18.6
13	YCO–Lime	20.7	16.5	18.6
14	YCO–Lime	21.7	16.9	19.3
15	Bordo–Tribasic	16.8	14.8	15.8
16	Bordo–Tribasic	17.0	15.6	16.3
Least difference needed for significance	Odds 19:1	3.5	3.5	2.5
	Odds 99:1	4.7	4.7	3.4

inclusive, gives some interesting information. There were 50 or more days with 0.01 inches of rain or more during these 4 months of the years when visible symptoms of copper injury appeared early in the season while there were only 38 and 35 such days in 1941 and 1944, respectively, when the early appearance of noticeable injury did not occur (Table 7). This indicates that wet weather is probably the principal factor influencing the occurrence of copper injury on grapes.

DISCUSSION

The following neutral copper fungicides have been found to cause injury to Concord grapes when used at a copper concentration equal to that in Bordeaux mixture 3–3–100: tribasic copper sulfate, copper compound A (4), Basicop (4), Yellow Cuprocide and Cuprocide 54-Y (4). Other neutral coppers were not tested. Bordow, which is a powdered Bordeaux mixture did not cause any significant injury (4). Throughout the various experiments on the control of grape diseases (5) after 1940, the neutral coppers were used at a copper concentration equal to Bordeaux mixture 2–2–100, or if used equal to Bordeaux mixture 4–4–100, there was 1 lb. of hydrated lime added to the spray mixture for each ¼ lb. of actual copper present in 100 gallons. In no case did apparent copper injury occur

TABLE 7.—*Records of rainy days*[a] *and rainfall*

Location (County)	Year	May		June		July		August		Total	
		Rainy days	Rain-fall	Rainy days	Rain-fall	Rainy days	Rain-fall	Rainy days	Rain-fall	Rainy days	Rain-fall
		No.	*Inches*	*No.*	*Inches*	*No.*	*Inches*	*No.*	*Inches*	*No.*	*Inches*
Seneca	1940	17	5.35	13	4.37	13	3.13	11	2.52	54	15.37
Chautauqua	1941	12	1.68	9	2.61	10	4.04	7	3.16	38	11.49
Do	1942	19	5.57	11	1.48	15	5.59	12	8.88	57	21.52
Do	1943	20	4.06	7	2.64	10	2.48	13	3.49	50	12.67
Do	1944	11	2.76	10	3.47	7	0.82	7	2.36	35	9.41

[a] Rainy Days=Days with 0.01 inches of rain or more.

with either of these spray mixtures. If neutral coppers are to be used alone to give a non-alkaline spray mixture, there should not be more than ½ pound of actual copper per 100 gallons of spray mixture (5). As shown in the experiments reported herein, the copper injury can be practically eliminated by the addition of the hydrated lime to the neutral coppers when they are used at a higher copper concentration in the spray mixture. S. E. C. oil was used as a spreader-sticker in the spray mixture when copper injury was first observed. There was no difference in the amount of copper injury that occurred when either rosin oil soap or S. E. C. oil were used.

Contrary to what might be expected, there was no significant difference in the yield from vines sprayed with Bordeaux mixture 2–2–100, 4–4–100, or 8–8–100. All of the Bordeaux plots showed a significant reduction in the 4-year average yield compared to that of the check. If the application I or applications I and II were omitted when Bordeaux mixture 8–8–100 was used, there was no significant difference in yield as compared to the check. The yield was not significantly greater than when the applications were not omitted.

Since the visible copper injury symptoms in the neutral copper plots

usually appeared by the blooming period, it is indicated that most of the injury occurs when applications I and II are made. However, in two treatments Bordeaux mixture was used for applications I and II and tribasic copper sulfate at 2–100 for applications III and IV. The yields from these treatments were reduced in the same order as the yields from the treatments which received the neutral copper without lime throughout the season.

In general the neutral coppers gave significantly more injury than Bordeaux mixture. When hydrated lime was added to the neutral copper the injury was alleviated and there was no significant difference in yield between plots with this spray mixture and Bordeaux mixture or the non-sprayed plots. In order of decreasing injury, the spray treatments ranked as follows: neutral coppers, Bordeaux mixture, neutral coppers plus lime, and non-sprayed. The weather appeared to be a contributing factor as to the presence and severity of copper injury on Concord grapes.

The effect of the fungicides on yield was obtained in a vineyard where no diseases were present. If diseases had been present, the yield from the sprayed plots would have been greater than the yield from the non-sprayed plots.

SUMMARY

Following the observation in 1940 that neutral copper fungicides used at copper concentrations equal to that of Bordeaux mixture 4-4-100 would cause injury to Concord grapes, a four-year investigation was made to determine the factors involved.

Copper injury caused by neutral copper fungicides is characterized by small, yellowish leaves, reduced cane growth, and reduced yield. With Bordeaux mixture the reduction in size of the leaves and the change in color were not apparent.

Tribasic copper sulfate, copper compound A, Basicop, yellow cuprocide and cuprocide 54-Y were the neutral coppers used and all of them caused injury (5). In the 4-year experiment, tribasic copper sulfate and yellow cuprocide caused a significant reduction in cane growth, yield, and size of bunches.

The injury caused by the neutral coppers was alleviated if they were used at a concentration of not over one-half pound of metallic copper per 100 gallons, as previously reported (5), which would be a neutral spray mixture, or at higher concentrations if one pound of hydrated lime was added for each one-fourth pound of metallic copper per 100 gallons.

Bordeaux mixture caused a significant reduction in yield although visible symptoms of copper injury were not present. There was no significant difference between the yields from plots sprayed with 2-2-100, 4-4-100, or 8-8-100 concentrations of Bordeaux mixture.

The amount of copper injury that occurred was not influenced by either the rosin fish oil soap or the S.E.C. oil used as a spreader-sticker.

The spray treatments would rank in order of decreasing injury as follows: neutral coppers, Bordeaux mixture, and neutral coppers plus lime.

One factor contributing to the severity of the copper injury was the number of days with 0.01 inches of rain or more during May, June, July, and August.

There were no diseases present in the vineyard at any time during the course of the investigation.

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RESISTANCE TO SPOTTED WILT IN TOMATO

FRANCIS O. HOLMES

Spotted-wilt disease of the tomato (*Lycopersicon esculentum* Mill.) has been observed by the writer occasionally in past years in New Jersey and neighboring states. It has not appeared to be a very common disease nor has a locally severe outbreak been recognized until recently. The reason for the infrequency of its occurrence has not been obvious, for elsewhere in the world, notably in Australia (5), in New Zealand (2), and in the Union of South Africa (4), spotted wilt has proved disastrous in its effects on tomato cultivation.

The disease known as ringspot of dahlia (*Dahlia pinnata* Cav.), studied originally by Brierley (1), has yielded a virus similar to that from spotted wilt of tomato in greenhouse and laboratory tests. Infected plants of dahlia are a probable source of much of the tomato disease. The causative virus can overwinter in dahlia corms whereas it usually cannot overwinter in tomato, a short-lived perennial species that is grown in the New Jersey area commonly as an annual crop and to a much less extent as a winter crop in greenhouses. Ringspot of dahlia, like spotted wilt of tomatoes, has been a relatively unimportant disease in the past in the eastern United States.

An unexpectedly severe outbreak of ringspot was observed in an extensive planting of dahlias near Vineland, New Jersey, in 1946. In this planting, slightly more than 30 per cent of the plants were obviously affected by October 10. Probably additional plants were infected but were not readily recognized as diseased at the time. Tomato plants had failed that year, and for a few years preceding had been difficult to grow, in the immediate neighborhood.

A study was made of the affected dahlia plots with a view to learning why ringspot disease, which is believed to be spotted wilt as it occurs in dahlia, should be so common there and what factors may have influenced an unprecedented spread to tomatoes. A third plant was found to be involved conspicuously. Chickweed (*Stellaria media* L.) was very abundant throughout the dahlia planting. It showed many white or yellow circular spots on old leaves and some distorted young leaves, such as had not been seen elsewhere. From affected chickweed leaves, a virus comparable to that isolated from ringspot lesions in dahlia was obtained by subinoculation to plants of *Nicotiana glutinosa* L. and tomato. Moreover the disease on chickweed could be reproduced readily by mechanical inoculation of healthy chickweed plants with virus originally derived from ringspot lesions in dahlia. Despite the potentially wide host range recognized for spotted-wilt virus (*Lethum australiense* H.), no other weed in the dahlia

fields appeared to be affected. There were present, however, abundant thrips, presumably including the ubiquitous onion thrips, *Thrips tabaci* Lind. (Thripidae), a vector of spotted wilt.

The severity of the local outbreak of the disease can be accounted for largely on the basis of extensive dahlia culture during the months of June to October, in an area heavily infested with chickweed, a winter annual occurring most abundantly from September to July. The seasons for growth of dahlia and chickweed overlap and together encompass the entire year. Transfer of virus from infected chickweed would tend to increase the incidence of spotted-wilt lesions in dahlia each summer; return of virus to seedling chickweeds in the autumn would complete a cycle. Chickweed alone could not be expected to maintain the disease, because the weed does not persist through the summer season in any quantity; and, in fact, fields a few miles removed from dahlia culture had only healthy chickweed plants. By March of 1947 the chickweed in the dahlia-growing area had been destroyed by intensive cultivation except in a small plot. The plants in this plot were so numerous as to touch each other over most of the surface of the ground. Almost all were diseased. Shortly afterward these also were destroyed with a view to attempting control of the disease. Had they been left to grow until July, much renewed spread of disease to dahlia might have been anticipated.

Varieties of tomato resistant to spotted wilt in the Territory of Hawaii had become available recently through the work of Kikuta, Hendrix, and Frazier (3), who developed the Pearl Harbor tomato and several improved derivatives of this variety. The writer had observed the adequacy of resistance of the Pearl Harbor tomato in Honolulu. It seemed possible that the outbreak of disease in New Jersey might be controlled, so far as tomatoes were concerned, by the use of such resistant lines. The Hawaiian investigators kindly supplied suitable seeds for this experiment.

Unfortunately, it was found that the New Jersey disease overcame the type of resistance that is characteristic of the Pearl Harbor tomato, both under conditions of natural spread in the field and under experimental conditions in the greenhouse. In the field a partial but insufficient resistance was displayed (See table 1). In the greenhouse, where infection was more nearly simultaneous and hence involved on the average younger plants and where dosage of virus was probably greater, hardly a trace of resistance was noted in the Pearl Harbor tomato and its derivatives. All inoculations in the greenhouse were made by mechanical means, that is, by rubbing leaflets in the presence of freshly expressed extracts of 10 to 12-day-diseased tomato plants and fine (320-mesh) carborundum powder.

Fortunately, seeds of a large number of unusual varieties of tomato were available in the writer's seed collection at the time; many were grown and seedlings produced from them were tested by mechanical inoculation. The virus used to test them was isolated originally from the dahlia by sub-

inoculation to *Nicotiana glutinosa* and subsequent transfer from the characteristic necrotic-type lesions on this plant to seedlings of Bonny Best tomato, a fully susceptible variety. In tomato, it produced tip blight symptoms and other manifestations characteristic of the tomato malady in the field. In expressed juice this virus was completely inactivated in 10 minutes at 45° C. To maintain the virus at sufficiently high titer for use in testing seedlings, subinoculations were made at approximately 10-day intervals to new young tomato plants. Tests of the virus when thus regularly transferred showed sufficiently high infectivity to produce, in one test, an average of 42.8 lesions per leaf in 10 leaves of *N. glutinosa*.

Most of the varieties tested proved highly susceptible but two outstandingly resistant lines of tomato were found.

One of the resistant tomatoes was known as Rey de los Tempranos (King of the Earlies). It was an Argentinian variety originally obtained

TABLE 1.—*Comparison of tomato varieties in the field at Vineland, N. J., on July 15, 1947*

Variety	Number of plants			
	Dead	Severely diseased	Moderately affected	Apparently healthy
Rutgers	26	10	4[a]	6[a]
Rey de los Tempranos	0	0	7	83
Pearl Harbor	17	40	6	27

[a] Only plants in the last two columns, moderately affected and apparently healthy, had produced appreciable amounts of fruit.

through the kindness of Dr. M. F. Babb, of the Cheyenne Horticultural Field Station in Cheyenne, Wyoming. All plants of this variety seemed to be resistant (See figure 1). Many of them showed no obvious response to inoculation, though a little virus could be recovered sometimes from inoculated leaflets, indicating localized and inapparent infection. A few became diseased systemically, but later sent out healthy branches and produced fruits. Seeds from a fruit on such an injured plant were grown. A progeny of 56 plants produced from them seemed as resistant as the variety itself, only two being moderately affected, whereas all of 44 plants of the susceptible variety Bounty, grown as controls, became systemically diseased. In the field, resistance proved to be of a similarly high order; a few plants became affected but the planting as a whole appeared vigorous and fruitful, whereas neighboring plantings of the varieties Rutgers and Pan America were so severely affected as to produce no usable fruits.

The second variety of value was the Manzana (Apple) tomato. This was another Argentinian variety, received through the kindness of Dr. F.

Rosenbusch of Buenos Aires. In this stock, the seeds of which were 10 years old when tested, some inhomogeneity existed. Part of the plants proved susceptible, but progenies that seemed wholly resistant were grown from seeds of resistant individuals.

First-generation hybrids between the susceptible Rutgers tomato and the resistant Rey de los Tempranos were not so resistant as the variety Rey de los Tempranos nor so readily infected systemically as plants of the seed parent, Rutgers. The same was true of a similar cross having Bounty as susceptible parent, instead of Rutgers, and of the reciprocal hybrid between Rey de los Tempranos, used as female parent, and Bonny Best, a
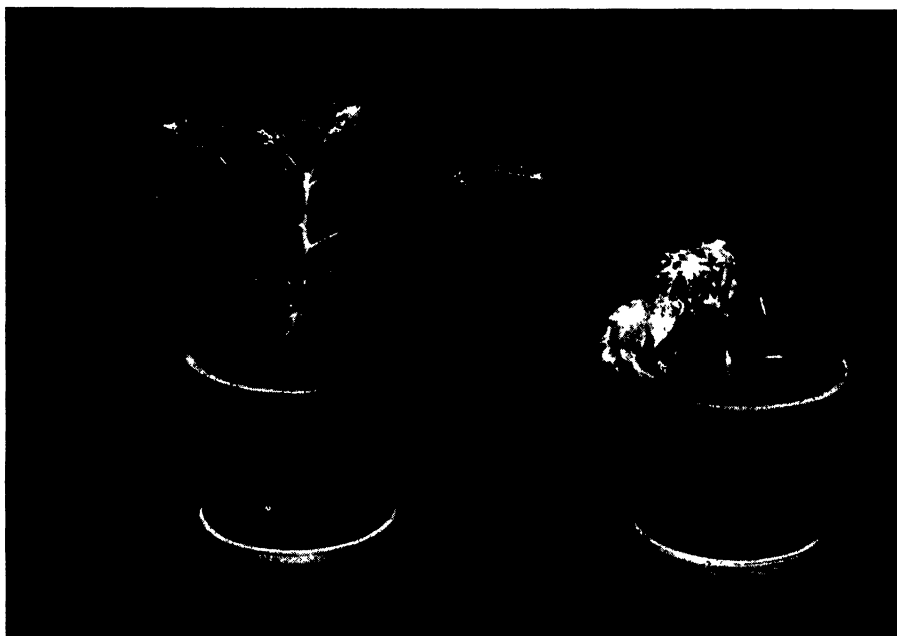
Fig. 1. An unaffected tomato plant of the resistant variety Rey de los Tempranos and a severely injured plant of the susceptible variety Rutgers, each 17 days after inoculation with spotted-wilt virus originally isolated from ringspot of dahlia in New Jersey. (Photograph by J. A. Carlile.)

fully susceptible sort, used as male parent. In all cases the F_1 plants seemed intermediate between the parental types in their responses to inoculation.

In a second generation of the Rutgers cross, grown from seed produced by self-pollination of flowers on the F_1 plants above mentioned, typical spotted-wilt disease was established in 299 plants of 401 inoculated; the remaining 102 plants reacted like the resistant variety Rey de los Tempranos, only 18 becoming somewhat affected later and these tending to send out healthy branches and to produce fruits. All of 397 inoculated control plants of the susceptible variety Rutgers became systematically diseased. The ratio of typically diseased to resistant plants among the second genera-

tion hybrids, 299:102, suggested that segregation for resistance had occurred in approximately a 3:1 ratio. A single-gene difference thus seemed to account for much if not all of the essential distinction between the resistant and susceptible stocks used in this test. The gene for resistance may be treated as though it were fully recessive in breeding experiments, although F_1 heterozygotes were shown to be more difficult to infect systemically than were the susceptible parent plants.

The Manzana tomato, as represented by selected resistant lines, was found to be as resistant as the variety Rey de los Tempranos in subsequent greenhouse and field tests in New Jersey. Manzana fruits were nearly spherical and of large size; they were red in color and of good flavor. Their only obvious defect was a tendency to concentric cracking and somewhat delayed ripening near the stem end of each fruit. Fruits of the variety Rey de los Tempranos were far more numerous and earlier in ripening, but were relatively small, flattened, and ridged.

The high degree of resistance of the variety Rey de los Tempranos in the field, the extreme susceptibility of the Rutgers tomato, and the intermediate but insufficient resistance of the Pearl Harbor tomato under New Jersey conditions are well shown in table 1.

Through the kindness of Mr. K. Kikuta and Dr. W. A. Frazier of the Agricultural Experiment Station at the University of Hawaii in Honolulu, Territory of Hawaii, the varieties found resistant in the field under New Jersey conditions were exposed to natural infection in Honolulu, on the grounds of the Agricultural Experiment Station, where a special test plot has been maintained for a study of resistance to Hawaiian spotted-wilt virus. Both Rey de los Tempranos and Manzana tomatoes appeared somewhat less resistant to spotted wilt there than the locally adapted resistant variety Pearl Harbor but much more resistant than the susceptible varieties Rutgers and Bounty. If the results of the preliminary test should be confirmed, this might indicate that the two types of resistance could be combined to advantage by crossing Pearl Harbor with Rey de los Tempranos or Manzana tomatoes. Among segregating progenies, some lines capable of resisting an increased variety of strains of spotted wilt might be found.

DISCUSSION

Spotted wilt is a viral disease of world-wide distribution. It has reached every continent and many oceanic islands. Infection seems to be much more frequent in some areas than in others, probably in accordance with availability of suitable plant hosts to act as reservoirs and thrips to act as vectors of the causative virus. Measures for control of the disease have sometimes been based on isolation of crops from cultivated or weed hosts and sometimes on destruction of insect vectors. Until recently no success has been reported in the search for immune or highly resistant varieties.

The first adequate resistance in tomato to damage by locally-occurring strains of the virus was reported in 1945 by the Hawaiian group of investigators, Kikuta, Hendrix, and Frazier (3). These investigators introduced the Pearl Harbor tomato and several excellent derivatives, which are capable of withstanding the disease as it occurs in the Hawaiian Islands. The existence elsewhere of one or more additional strains of virus requiring another type of resistance in tomato is illustrated in the present investigation. Perhaps the virus will continue to disclose potentially dangerous strains, for which the now known types of resistance may be inadequate. Further selection of types of resistance may be needed to complete eventually the solution of the world-wide problem of control of spotted wilt in tomato.

Comparison of experiences in the Hawaiian Islands with subsequent studies in New Jersey teaches a somewhat unexpected lesson. Investigators in the Hawaiian Islands might logically have concluded that reintroduction of spotted-wilt virus would be without effect, because the Islands long have been infested by strains of spotted-wilt virus and in recent years have possessed also an adequate resistance in tomatoes. If spotted-wilt virus from New Jersey had been introduced, however, the adequacy of existing resistance in tomatoes would have been severely tested and perhaps nullified, unless the damaging new strains had found conditions there unsuited to their multiplication or transmission. Fortunately, adequate resistance for New Jersey strains of virus is now available if needed. The insight provided by tests of the Pearl Harbor type of resistance in New Jersey would suggest, however, that is not advisable to introduce a virus into a country, especially one normally protected by ocean barriers, even though strains of the virus are reported as present already.

In general it has been supposed that a virus strain is capable of giving rise to all possible derivatives wherever it exists. There may, however, be practical limitations to selection and establishment of some variants. If mutation of one strain gives rise to a derivative especially well suited to potential host plants in an environment, the derived strain may persist and even become dominant. This evolutionary process may go on through a series of steps. Conceivably it may also reverse itself, so far as mutation is concerned, but suitable hosts to support the whole chain of events required for a gross change in characteristics or for a stepwise return may not be available, especially in a limited environment, such as that of an island or a small group of islands. For this reason certain strains may not be developed, selected, and established locally.

It will be of interest to test the two now known types of resistance in all the areas of the world from which spotted-wilt disease has been reported. Will the first-described virus of spotted-wilt, that reported from Australia by Samuel, Bald, and Pittman (5), resemble that of the Hawaiian Islands, that of New Jersey, or neither of these, in its effect on tomato plants bearing the Pearl Harbor type and on those bearing the newly described type of

resistance? Will the so-called kromnek disease in the Union of South Africa (4) resemble spotted-wilt disease as first recognized in Australia in its relationship to the two types of resistance? Whereas it would be obviously unsafe to import spotted-wilt viruses from different continents for comparison with one another, it would seem to be a safe and expedient process to send seeds of resistant tomatoes to all affected areas.

SUMMARY

An outbreak of spotted-wilt disease in a small area of southern New Jersey was caused by a strain or strains of the causative virus capable of overcoming the resistance of the Pearl Harbor tomato and its recent derivatives as developed in the Hawaiian Islands. A heritable resistance adequate for control of the New Jersey disease was found in two kinds of South American tomato, the variety Rey de los Tempranos and some lines of the variety Manzana, both from Argentina. The new type of resistance, as exemplified in the variety Rey de los Tempranos, was inherited as a single Mendelian factor in crosses with the susceptible Rutgers variety of tomato; approximately one quarter of all plants (102 of 401) in the second hybrid generation displayed resistance comparable to that of the resistant parent.

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EFFECTIVENESS OF FUNGICIDES IN CONTROLLING BLACK ROT OF SWEET POTATOES

L. H. PERSON, E. O. OLSON, AND W. J. MARTIN[1]

In recent years black rot of sweet potatoes caused by *Ceratostomella fimbriata* (E. and H.) Ell. has been assuming greater importance in the commercial sweet potato areas in Louisiana. This situation is attributed to a general relaxation in the use of sanitary practices necessary for the control of the disease and to the introduction of the practice of washing potatoes immediately before packing and shipping. Black rot has caused some losses in stored, unwashed potatoes, but the greatest losses have occurred during transit to market in the early crop which is harvested and shipped in July and August. Affected potatoes as they reach the market are illustrated in figure 1, A.

A fungicide which would be effective against black rot and could be used without harmful effects on the freshly dug sweet potatoes after washing, would be of considerable value in preventing losses from this disease. Some attempts have been made to find such a fungicide. Daines[2] and Parris[3] have reported on the use of borax in the control of soft rot of sweet potato and Jeffers[4] has reported on tests of several fungicides for preventing infection of sweet potato slices by *Ceratostomella fimbriata*.

During the past five years a number of fungicides have been tested in Louisiana for their effectiveness in controlling black rot. The results of these tests are reported in this paper.

MATERIALS AND METHODS

The sweet potato variety Porto Rico was used throughout and all tests with the fungicides were made in essentially the same way. After the sweet potatoes were washed they were dipped in a heavy spore suspension of *Ceratostomella fimbriata* and then immersed for about 30 seconds in a water solution or suspension of the fungicide to be tested. The potatoes were then placed in large paper-lined crocks or crates. To serve as checks potatoes were dipped in the spore suspension, placed in crocks or crates and stored under the same conditions as the treated potatoes. After a period of approximately 15 days the potatoes were examined. Counts were made of the lesions on each potato and the average number of lesions per potato in each treatment was calculated.

[2] Daines, R. H. Soft rot of sweet potatoes and its control. New Jersey Agr. Exp. Sta. Bul. 698. 1942.

[3] Parris, G. K. Control of soft rot of freshly washed and waxed, uncured sweet-potatoes due to *Rhizopus nigricans*: A preliminary report. U. S. Dept. Agr., Pl. Dis. Rptr. 28: 1168–1169. 1944.

[4] Jeffers, W. F. Evaluation of several fungicides in preventing infection of sweet potato slices by *Ceratostomella fimbriata*. (Abstr.) Phytopath. 37: 439. 1947.

TABLE 1.—*Results of tests showing the average number of black-rot lesions per potato which developed on the treated and untreated lots*

Treatment		No. of tests	No. of potatoes used		Ave. no. of lesions per potato	
Fungicide[a]	Concentration[b] (Per cent) in water		Treated	Untreated	Treated	Untreated
Borax	2.50	1	22	22	0.5	8.0
	1.25	7	124	127	0.9	8.4
	1.00	1	19	24	0.9	5.4
	0.67	1	24	24	0.8	5.4
	0.63	5	79	79	1.2	10.4
	0.50	1	21	24	1.3	5.4
	0.31	2	21	20	4.8	11.0
Dithane D–14	1.00	3	75	73	0.4	5.8
	0.67	4	106	105	0.6	6.6
	0.50	3	51	51	0.9	5.3
	0.25	3	36	37	4.2	9.1
	0.13	1	10	10	11.5	19.5
Acetic Acid	1.00	1	10	10	4.9	13.4
Calcium hypochlorite	Saturated	1	11	10	12.5	13.4
Dithane Z–78 . .	0.20	1	41	39	1.0	6.8
	0.10	1	39	39	1.5	6.8
Dowicide A . ..	0.40	1	18	17	12.1	13.1
Dowicide B . .	0.40	1	16	17	.4	13.1
Elgetol	0.67	1	9	10	5.2	13.4
8-Hydroxyquinoline sulfate	1.00	1	15	17	5.0	13.1
Isothan Q–15	0.50	1	16	17	10.7	13.1
Mercuric chloride .	0.10	2	53	53	0.5	7.1
Phygon	1.50	1	40	39	1.4	6.8
	0.30	1	40	39	2.7	6.8
	0.18	4	42	40	2.6	9.1
	0.09	2	20	20	2.5	11.0
	0.05	1	10	10	5.7	19.5
Potat-O-Dip	0.60	2	53	53	5.5	7.1
Puratized N5E ⋅	0.50	3	37	37	1.0	10.3
Puratized 177 . .	0.25	1	10	10	8.6	13.4
Puratized N5D . ..	0.20	1	22	22	0.1	8.0
Thiourea	1.50	3	31	30	1.2	7.6
	0.75	2	20	20	4.7	11.0
	0.38	2	20	20	5.3	11.0
	0.19	2	19	20	5.5	11.0

[a] The borax used was 20 Mule Team Brand made by Pacific Coast Borax Company; Dithane D–14 is a liquid containing 25 per cent disodium ethylene bisdithiocarbamate; Dithane Z–78 is zinc dimethyl dithiocarbamate; Dowicide A is sodium o-phenyl phenate; Dowicide B is the sodium salt of 2,4,5-trichloro phenol; Elgetol is sodium dinitro-ortho-cresol; Isothan Q–15 is lauryl isoquinolinium bromide; Phygon is 2,3-dichloro-1,4-naph-thoquinone; Potat-O-Dip is a powder containing not less than 31.0 per cent aromatic phenylphenols and 0.1 per cent aromatic hydroxynitrocresols; Puratized N5D (10 per cent active agent) and Puratized N5E (5 per cent active agent) are phenylmercuritri-ethanol ammonium lactate; Puratized 177 (20 per cent active ingredient) is para amino phenyl cadmium dilactate.

[b] Concentration was based on the commercial product or experimental sample used and not on active ingredients.

TESTS WITH FUNGICIDES

Seventeen compounds were tested and the results with these are summarized in table 1. Since the tests were carried out over a long period of time and under different conditions, the results with each compound are given in comparison with the checks for the tests in which the material was used.

Based on the average number of lesions which developed on the treated compared to the untreated sweet potatoes, the following compounds are considered as having given satisfactory control of black rot: (1) borax at 0.63 per cent or stronger, (2) Dithane D–14 at 0.5 per cent or stronger, (3) Dithane Z–78 at 0.2 per cent, (4) mercuric chloride at 0.1 per cent, (5) Phygon at 1.5 per cent, (6) Puratized N5E at 0.5 per cent, (7) Puratized N5D at 0.2 per cent and (8) thiourea at 1.5 per cent. Since mercuric chloride and the two Puratized compounds contain mercury, they should not be used for treating potatoes that are for human consumption until further analyses are made; but they are safe for use in surface-treating roots before

TABLE 2.—*The average number of black-rot lesions developed on inoculated sweet potatoes and on similar potatoes treated in different concentrations of borax*

Treatment	No. of potatoes used	Ave. no. of lesions per potato
Untreated—checks	39	6.8
Borax, 1.0 per cent	38	0.1
0.7	40	0.1
0.5	40	0.1
0.3	40	0.4
0.1	39	0.5

bedding them for slip production. The Dithane Z–78, Phygon, and thiourea treatments were somewhat less effective than the borax and Dithane D–14 treatments. Thus, borax and Dithane–14 seem to have the best possibilities for use in treating sweet potatoes for the prevention of black rot after the commercial washing process, provided the solutions are allowed to dry on the surface. Preliminary tests indicate that the borax treatments are not effective if the potatoes are washed in water after treatment. Borax-treated and untreated potatoes are shown in figure 1, B and C.

EFFECT OF WEAK SOLUTIONS OF BORAX

To determine the lower limits of concentration of borax effective in preventing the development of black rot on inoculated sweet potatoes, a test was made with different concentrations from 0.1 per cent to 1.0 per cent. The results of this test are summarized in table 2. Concentrations as low as 0.1 per cent borax gave striking reductions in the average number of black-rot lesions which developed per potato.

TESTS WITH MARKET SWEET POTATOES

In a test under commercial conditions approximately 2400 potatoes showing no evidence of black rot were obtained from a lot of potatoes which
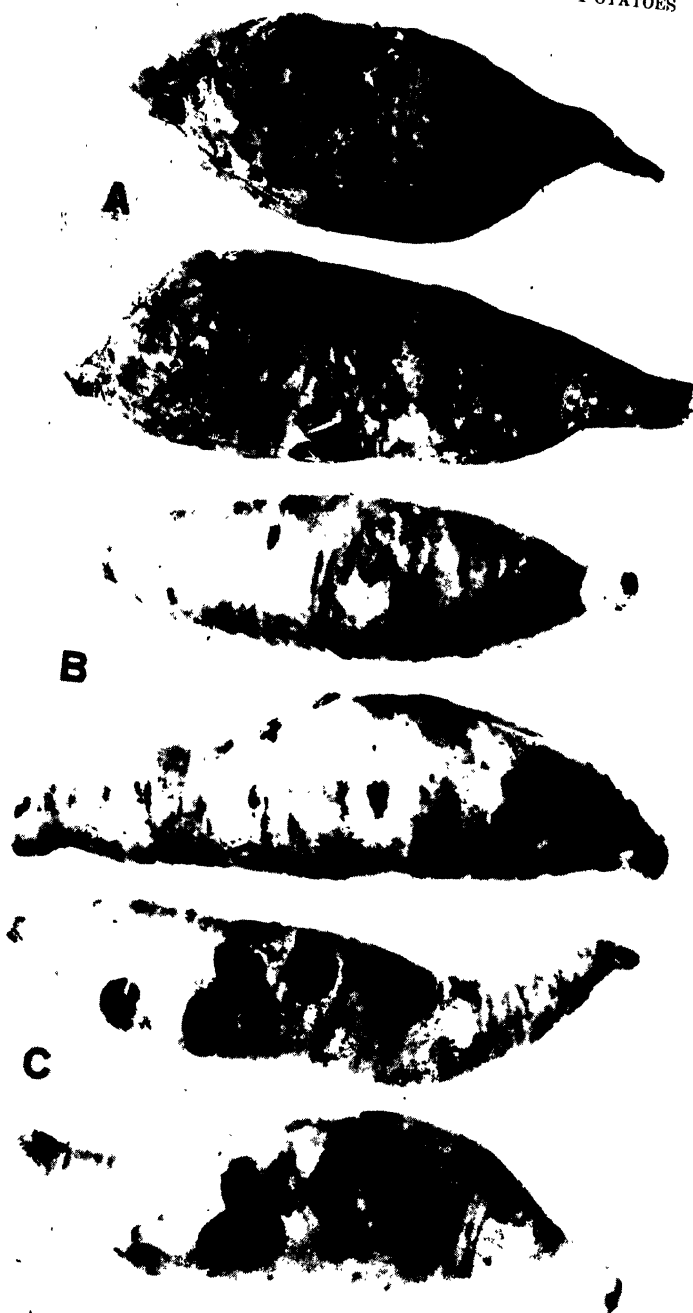
FIG. 1. A. Sweet potatoes which have developed black rot during transit to market.
B. Sweet potatoes treated in a one per cent solution of borax after inoculation with
Ceratostomella fimbriata. C. Sweet potatoes not treated after inoculation.

had some black rot in them at the time of washing. Immediately after the potatoes came off the washing machine, they were divided into four groups, one for each of the following treatments: (a) untreated checks, (b) dipped in a 1.3 per cent solution of Dithane D–14, (c) dipped in a 1.0 per cent solution of borax, and (d) dipped in a 0.6 per cent solution of borax. The potatoes were stored in paper-lined crates as for shipment and notes were taken two weeks later. The average number of lesions which developed per potato in the four treatments were: (a) 0.25, (b) 0.03, (c) 0.03, and (d) 0.05.

INJURY TO THE SWEET POTATOES RESULTING FROM TREATMENTS

In the borax treatments some chemical burning was observed on potatoes treated in 2.5 per cent solutions (Fig. 2, A). No serious burning was ob-

FIG. 2. Sweet potatoes showing chemical burn after treatment for control of black rot. A. Treated in a 2.5 per cent solution of borax. B. Treated in a 1.3 per cent solution of Dithane D–14.

served with any of the other concentrations of borax used. In the test with market potatoes the potatoes used were rather badly skinned and bruised at the time of treatment but even under these conditions the potatoes treated in 0.6 and 1.0 per cent solutions of borax showed no evidence of burning.

In the treatments with Dithane D–14, a rather severe chemical burning was noted on the bruised potatoes treated in 1.3 per cent solution (Fig. 2, B). Under laboratory conditions no evidence of burning was noted with concentrations up to 1.0 per cent of Dithane D–14.

BORAX RESIDUES

In order to have information concerning borax residues on treated sweet potatoes, Dr. E. A. Fieger of the Department of Biochemistry, Louisiana

State University, analyzed potatoes which had been dipped in solutions of borax at concentrations of 1.25 and 0.63 per cent. This was done to determine if borax in sufficient amounts adhered to the potatoes to make them unsafe for human consumption. The results of these analyses, which are included in table 3, indicate that boron residues are small.

TABLE 3.—*Results of analyses of sweet potatoes treated with borax*

Potatoes analyzed	Dry weight of boron (p.p.m.) in potatoes		
	Untreated	Dipped in 0.63 per cent solution of borax	Dipped in 1.25 per cent solution of borax
Raw (unwashed)			
Peelings	15.5	172.60	445.20
Flesh	4.8	4.40	6.70
Whole	4.8	18.40	33.20
Raw (unwashed) stored 20 days			
Peelings	16.28	157.90	285.70
Flesh	4.27	4.46	7.24
Raw (washed) whole			
In stream of water	4.80	17.50	22.20
With brush	4.80	13.40	22.40
Cooked (flesh)			
Baked	4.50	7.20	3.40
Peeled then boiled	3.70	7.00	4.90
Boiled in skins	4.20	6.30	5.30

SUMMARY

In Louisiana, black rot of sweet potatoes has increased in importance in the early crop which is washed and shipped to market before curing. In an attempt to find a fungicide treatment for use on these potatoes after the commercial washing process, 17 compounds were tested for their effectiveness in controlling black rot. The results of these tests indicate that there are definite possibilities for the commercial use of borax and perhaps Dithane D–14 at concentrations of approximately one per cent for controlling black rot on uncured sweet potatoes.

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INFLUENCE OF SOIL MOISTURE ON THE GROWTH OF THE POTATO PLANT AND ITS INFECTION BY THE ROOT-KNOT NEMATODE

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The potato (*Solanum tuberosum* L.) has been grown as a commercial crop in the Territory of Hawaii since 1933, but not until the 1937–38 season was severe galling of tubers by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, noted. This season was outstanding in that rainfall was higher than usual and the soil remained wet for much of the time from December to April, when potatoes are produced in Hawaii.

Godfrey (1) showed that the amount of moisture in the soil seemed to play only a small part in root-knot development as long as the moisture content was favorable to the growth of crops. Little difference was found within the range of 40 to 80 per cent of the moisture-holding capacity of the soil; at 60, 70, and 80 per cent there appeared to be a slight increase over other moisture percentages but below 40 and above 80 per cent considerable knotting was found. Godfrey was of the opinion that temperature played a more important part than soil moisture in the development of *Heterodera marioni*. Gross (4) has reported an interesting observation: potatoes grown on land irrigated at frequent intervals showed less tuber galling than potatoes grown on land of equal infestation but irrigated less often. He believed that lowering the soil temperature from frequent irrigation inhibited the development of *H. marioni*. When controlled irrigation was tried there was but slight reduction in visible symptoms however, and daily irrigation of land caused a reduction in soil temperature of 2.7° F., compared with the temperature of soil irrigated at 5–day intervals.

The data presented here were obtained from greenhouse tests designed to determine whether galling of tubers is more severe in wet soils than in soils of lower moisture content, and to measure the reduction in yield of potatoes due to (a) inadequate soil moisture, (b) *Heterodera marioni*, and (c) a combination of these. Two separate experiments were conducted; one in which potatoes were grown in field soil that was infested, naturally and also artificially, with *H. marioni;* and another in which potatoes were grown in infested and fumigated soils.

EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF HETERODERA MARIONI IN POTATO TUBERS AND ROOTS

Materials and Methods

In this experiment, which lasted from August 1 to October 28, 1939, the

air temperatures in the greenhouse were 83° to 93° F. (weighted mean 87.7° F.) during the day, and 64° to 75° F. (weighted mean 70.5° F.) at night. For the two combined the weighted mean is 79.1° F., which is within the range reported by Godfrey (1) for gall formation. Soil collected from around the roots of field grown tomato plants affected with root-knot was mixed with finely chopped nematode infected roots of cucumber (*Cucumis sativus* L.) and bean (*Phaseolus vulgaris* L.). This soil was a reddish clay loam, of almost neutral reaction, with a field capacity moisture content of approximately 36 per cent, a moisture equivalent of approximately 32 per cent, and a wilting coefficient of approximately 24 per cent. The soil was well mixed daily for one week and kept moist. Commercial fertilizer considered adequate for normal growth of potato plants was incorporated, and an equal weight of soil was placed in each of 35 twelve-inch diameter clay pots previously weighed and counterbalanced. Their outer surfaces had been varnished to reduce evaporation. Several pieces of coarse wire screen were placed in the bottom of each pot for drainage. One potato tuber, 1.5-inch diameter, variety Bliss Triumph, outwardly free from nematode infestation was planted in each pot. Water was poured on the soil surface until leaching started, and also below the surface through 3 one-half inch diameter glass tubes whose lower ends were open and flared and placed 2, 4, and 6 inches deep, respectively. When leaching stopped the pots were weighed individually; although small differences in weight existed, the pots approximated 31 lbs. each. Soil moisture at this time, on an oven-dry basis, was 35 per cent, or almost the field capacity.

Fourteen pots were maintained at 31 lb. for 1 month by daily weighing and addition of water. The individual weights of 7 pots were allowed to fall to 29.5 lb., and those of the remaining 14 pots to 28 lb. by withholding water, which took about 1 week; these two weights were maintained for 1 month. For convenience in description, pots that weighed 31 lb. during the first month are referred to as the "Wet" series; pots that weighed 29.5 lb. during this time are referred to as the "Medium" series; and pots that weighed 28 lb. during the same period are referred to as the "Dry" series.

No claim is made in this experiment, or in experiment 2, that the moisture content of the soil in the series Wet, Medium, or Dry ever was held constant. Soil samples taken at intervals and at the end of the experiment showed that moisture in the soil probably fluctuated between 31.5 and 35 per cent in the Wet series, between 27.5 and 30 per cent in the Medium series, and between 24 and 27 per cent in the Dry series.

Seven pots were kept in each of the series (Wet, Medium, and Dry) for the duration of the experiment. Thirty days after planting 7 pots were transferred from the Wet series and 7 from the Dry series to the Medium series. To compensate for the growth of tops and roots, the maintained weight of every pot was increased by one-half pound. Sixty days after

planting, the 7 pots in the Medium series that had been transferred there at 30 days from the Wet series were placed in the Dry series, and the 7 originally in the Dry series were placed in the Wet series. When the first change in soil-moisture was made (30 days), plants previously in the Wet series when changed to the Dry series wilted, particularly during the day, but recovered at night. Wilting was less marked, or absent, when plants previously in the Wet series were changed to Medium. After about 5 days no wilting was observed.

Results

All plants when 30 days old were measured for height; those in the Wet series averaged 11.7 cm., those in the Medium averaged 8.9 cm., and those in the Dry were 7.3 cm.

In table 1 a summary is given of the relative vigor of tops of the potato plants, at 60 days, in relation to the different moisture treatments. From the "mean vigor scores" it can be seen that most vigorous growth was

TABLE 1.—*Relative vigor of potato plant tops in relation to soil moisture, after 2 months*

Soil moisture [a]	Number of plants per vigor class				Mean vigor score [b]
	Very good	Good	Poor	Very poor	
WW	6	1	0	0	3.9
MM	0	4	3	0	2.6
DD	0	2	2	2	2.0
WM	3	3	1	0	3.3
DM	0	4	1	2	2.3

[a] W = wet, M = medium, D = dry; the 2 letters represent moisture conditions for consecutive 30-day periods. Seven plants were in each group, but one plant in DD was lost through accident.

[b] Vigor scores: 1 = very poor, 2 = poor, 3 = good, and 4 = very good.

obtained when the soil was continuously wet (WW) and poorest when it was continuously dry (DD), with a somewhat intermediate figure when the moisture was moderate in its amount (MM). Growth was better in the WM series than in the DM, MM, or DD series.

Plants were harvested as soon as the tops died, usually 70 to 82 days after planting, with a few instances of 85 to 89 days. There was no evidence that the potato plants were attacked by organisms other than *Heterodera marioni*. Tubers, underground stems and stolons, and roots were washed free of soil, the tubers weighed, and tubers and roots examined, outwardly and microscopically, for *H. marioni*. Infection generally was heavy.[2] Data are summarized in table 2. Yields were highest in the WWW series, that is, with the soil kept continuously well moistened; but accompanying the high yields was severe galling of tubers and often of roots. Fifty per cent less yield, but with less galling of tubers and roots

[2] Eighteen Whippoorwill cowpea plants, 3 growing for 30 days in each of 6 small cans, each can containing approximately 650 grams of the soil in which the potatoes were planted had an average of 616 nematode galls per plant on the root systems.

and lowest mean severity scores, was obtained in the WMD series, in which the soil moisture was lowered progressively as the plants got older. Galling of tubers was less in moisture series MMM and DDD than in the WWW series, with most severe galling of roots in series DDD. Somewhat confirming the trend is the result found in series DMW, where galling of tubers was more severe than in series WMD. Findings in experiment 1 appear to show that tuber galling is more severe in wet than in dry soil, while root galling is more pronounced in dry than in wet soil.

TABLE 2.—*Longevity, yield, and severity of attack by Heterodera marioni of potato plants in infested soil when held at relatively constant levels of moisture or varied at monthly intervals*

Soil moisture [a]	Av. life of plants (days)	Av. yield (gm.)	No. plants with tubers		Mean severity scores	No. plants with roots		Mean severity scores [b]
			Severely galled	Slightly galled		Severely galled	Slightly galled	
WWW	81	320	7	0	2.0	5	2	1.7
MMM	71	100	4	3	1.5	5	2	1.7
DDD	70	24	4	2	1.6	6	0	2.0
WMD	79	161	2	5	1.2	2	5	1.2
DMW	70	95	7	0	2.0	6	1	1.6

[a] W = wet, M = medium, D = dry; the 3 letters represent moisture conditions for consecutive 30-day periods. Seven plants were in each group, but one plant in DDD was lost through accident.

[b] Mean severity scores: 1 = slightly galled, 2 = severely galled. Mean severity score represents sum of plants in each severity class times the score for that class divided by the total number of plants.

EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF HETERODERA MARIONI ON POTATO AND ON NORMAL GROWTH OF THE PLANT

Materials and Methods

To better interpret the results of the first experiment a second was planned. Potatoes were grown in nematode-infested and in fumigated soil and the effect of differences in soil moisture on the growth of the plant and on the activities of *Heterodera marioni* determined. The same soil type was used as in experiment 1 and it was handled similarly except that it was infested with peelings from the nematode-infected potato tubers from experiment 1. Enough soil was infested to later fill 60, twelve-inch diameter clay pots, varnished on their outer surfaces. Commercial fertilizer adequate for normal growth was mixed with the soil. Half of the soil was fumigated 5 days with chloropicrin (2.5 cc. per cubic foot) in closed containers, spread out on tables in the greenhouse for 5 days to allow escape of the nematocide, then well wetted, and placed in 30 pots. Pots were counterbalanced as before, an equal weight of soil placed in each, and one

potato tuber (Bliss Triumph), outwardly free of nematode infection, planted per pot. The soil was watered until seepage started; when it stopped the pots plus soil were weighed. Experiment 2 lasted from November 8, 1939, to February 5, 1940, when the greenhouse day temperatures varied from 74° to 99° F. (weighted mean 87.6° F.) and the night

TABLE 3.—*Longevity, yield, and severity of attack by Heterodera marioni of potato plants in infested and also in fumigated soil when held at relatively constant soil moistures or soil moistures varied at monthly intervals*

Soil treatment and soil moisture [a]	Age of plants when harvested (days)	Av. yield (gm.)[b]	No. plants with tubers		Mean severity scores[c]	No. plants with roots		Mean severity scores[c]
			Severely galled	Slightly galled		Severely galled	Slightly galled	
Fumigated								
WWW	30	0	.		.. .	0	0	0.0
	60	368	0	0	0.0	0	0	0.0
	90	550	0	0	0.0	0	0	·0.0
DDD	30	0	.	..	....	0	0	0.0
	60	214	0	0	0.0	0	0	0.0
	90	297	0	0	0.0	0	0	0.0
WMD	60	266	0	0	0.0	0	0	0.0
	90	388	0	0	0.0	0	0	0.0
DMW	60	200	0	0	0.0	0	0	0.0
	90	410	0	0	0.0	0	0	0.0
Infested								
WWW	30	0	...	...		0	2	0.6
	60	146	2	1	1.6	0	3	1.0
	90	324	2	1	1.6	1	2	1.3
DDD	30	0		..	.	0	1	0.3
	60	57	1	1	1.5	2 .	0	2.0
	90	152	1	2	1.3	3	0	2.0
WMD	60	180	1	1	1.0	0	3	1.0
	90	222	2	1	1.6	1	2	1.3
DMW	60	165	1	2	1.3	2	1	1.6
	90	245	3	0	2.0	2	1	1.6

[a] W = wet, M = medium, D = dry; the 3 letters represent moisture conditions for consecutive 30-day periods. Three plants were harvested at 30, 60, and 90 days, respectively, from each group except at 60 days in infested soil, group DDD, where one plant was lost through accident.

[b] Variation in yield between individual plants was less than 10 per cent; a yield of 0 indicates that no tubers were present.

[c] Mean severity scores: 0 = not galled, 1 = slightly galled, 2 = severely galled. Mean severity score represents the sum of plants in each severity class times the score for that class, divided by the total number of plants.

temperatures from 57° to 74° F. (weighted mean 65.4° F.); the weighted mean for all temperatures combined is 76.5° F., within the range reported by Godfrey (1) for gall formation.

The two extremes of soil moisture, Wet (W), and Dry (D), were studied; and in a few pots the soil was held at the Medium (M) moisture during the second month of the plants' growth. Pots in the Wet series were maintained at 34.5 lb. for 30 days after planting, increased to 35 lb. for the next 30 days, and after 60 days increased to 35.5 lb. In the Dry

soil-moisture level, saturated soil was allowed to dry out until weight of pot plus soil was 31 lb., which took about 5 days; the pots were maintained at 31 lb. for 30 days after planting, increased to 31.5 lb. for the next 30 days, and after 60 days increased to 32 lb. Medium soil moisture was obtained by keeping the combined weight of pot plus soil at 32.5 lb. The percentage of soil moisture in the Wet series on an oven dry basis varied from 32 to 36 per cent as shown by testing samples taken from various pots, and from various parts of the soil within individual pots, at intervals during the course of the experiment. In the Medium moisture series the soil moisture varied from 29 to 33 per cent, and in the Dry series the variation was 24 to 30 per cent. Four moisture schedules were followed: (a) Eighteen plants in soil continuously Wet (WWW); (b) Eighteen plants in soil continuously Dry (DDD); (c) Twelve plants in soil that was Wet for 30 days, moderately moist (M) for the next 30 days, and Dry for the next 30 days (WMD); and (d) Twelve plants in soil with moisture conditions the reverse of "c" (DMW). Half of the pots in each of these 4 schedules was filled with nematode-infested soil and the other half with fumigated soil. Pots were weighed daily and returned to desired weights by addition of water. Six plants, 3 growing in nematode-infested soil and 3 growing in fumigated soil, were harvested at 30, 60, and 90 days after planting, respectively, from moisture schedules WWW and DDD. Sixty days after planting, and again at 90 days, 6 plants, 3 growing in infested soil and 3 growing in fumigated soil, were harvested from moisture schedules WMD and DMW, respectively (Table 3).

Roots, tubers, and underground stolons were washed free of soil, and the tubers weighed. After macroscopic examination, the roots were discarded. Tubers next were examined, macroscopically and microscopically, for nematode infection; no infection was found on underground parts of plants grown in fumigated soil. Data are summarized in table 3.

Results

Highest yields were obtained in continuously Wet (WWW) fumigated soil and lowest in infested Dry (DDD) soil, at both 60 and 90 days. Plants harvested at 30 days bore no tubers. At 60 days, yields in Dry soil were reduced 42 per cent, compared with an 85 per cent reduction in infested Dry soil and a 60 per cent reduction in infested Wet Soil. At 90 days there was little difference in yield in a fumigated Dry soil and a Wet soil infested with *Heterodera marioni;* in the Wet soil the nema reduced yields by 41 per cent and in the Dry soil the reduction was 48 per cent. The lower the soil moisture the greater the reduction in yield attributable to *H. marioni.*

When the soil moisture was varied, results were not always so clear cut. In fumigated soil, moisture schedule DMW produced 24 per cent less tubers at 60 days than WMD and about the same as WMD at 90 days, but at

neither date did either DMW or WMD produce the yield obtained in WWW soil. In infested soil there was little difference between yields, at 60 or 90 days, in schedules WMD and DMW, and again neither produced the yields obtained in WWW soil (infested or fumigated). To obtain greatest yields in the presence of *Heterodera marioni* it seems necessary to have the soil moisture as high as possible.

Tubers were not attacked so severely as in experiment 1, possibly due to slightly lower air and soil temperatures. Infection was adequate, however, to show that tubers grown in wet soil are attacked more severely by *Heterodera marioni* than tubers grown in dry soil (Table 3); for tubers of the same size, from wet and from dry soil, there were more and larger galls on the former. In contrast, more root galling was encountered in dry infested soil, which was thought to be due to infection by *H. marioni*. No counts of galls per root system were attempted. These findings confirm those obtained in experiment 1.

Tubers produced in wet soil under field conditions usually have enlarged lenticels. In experiment 2 enlarged lenticels were found in fumigated and also in infested wet soil. Only by microscopic examination could the plant morphology, lenticel, lenticel-gall, or gall be determined with certainty. Freehand sections through the enlargements, killed and fixed in chromacetic acid and stained with osmic acid (2), sufficed to reveal the presence or absence of the nematode[3]. Lenticels seem to serve as avenues of entrance for larvae of *Heterodera marioni*, and it was easy to find larvae just beneath the cambial layer of enlarged lenticels, obviously recent infections. Large galls that had been originally lenticels were also observed, with the nematode, in various stages of development, lying directly opposite the natural opening of the epidermis of the tuber. That enlarged lenticels serve as avenues of entrance for *H. marioni* has not been pointed out before. Enlarged lenticels on tubers produced in wet fumigated soil showed no nematodes, and at the time it was not possible to examine lenticels of normal size for nematode infection.

DISCUSSION

These results are based on a relatively small number of plants in each series, but the nature of the research called for expending considerable time in handling the plants and a greater number, while desirable, was not possible. The method of adding water to the soil is open to criticism; each time water is added the wet soil condition is established locally, permitting the nematode and contaminating fungi to overcome any temporary limitations imposed by previous drought. Differences in soil moisture were obtained, however, the "wet" soil differed considerably from the "dry" soil, and to obtain these contrasts was the endeavor of the research. The

[3] No substitute for the expensive osmic acid was found though much time was spent trying alkannin, Sudan III, cotton blue, acid fuchsin, and scarlet R, the last 3 of which are recommended by Goodey (3) for differentation of *Heterodera marioni* in root and stem tissue. They did not work in potato tuber tissue.

nematode *Heterodera marioni* seems to injure potato tubers more in wet soil than in dry soil, certainly their sales value is lessened, but it also seems to cause less injury to roots in wet than in dry soil. Roots were galled severely, sometimes even rotted and decaying, in dry infested soil, whereas in wet infested soil the root systems seemed more capable of competing with the attacks of the nematode. It is well known that presence of moisture in the soil is an inducement to root formation. What are the relative speeds of root galling and new root formation in a wet soil and in a dry soil? If the nematode can attack and gall roots as soon as they are formed then the nematode will be dominant. If, however, a plant can produce roots faster than the nematode can attack them, the general picture will be one of a plant with functioning roots not suffering particularly from attack by *H. marioni*. Detailed research is needed, but the results obtained here indicate That in nematode infested soil, for good yields, the potato plant needs abundant soil moisture although galling is more severe and noticeable in wet soil than in drier soil. In dry soil the tuber galling is less than in wet soil but yields are low, probably lower than commercially desirable.

The evidence presented here is not in agreement with the observation by Gross (4) that potatoes grown on land irrigated at frequent intervals had less tuber galling than potatoes irrigated less, but a comparison between the two locales is hardly justified because too many other factors are operating. In fact, Gross admits that controlled irrigation resulted in only a slight reduction in visible symptoms; even in the same location it was difficult to duplicate results. These present data tend to confirm field results of the 1937–38 potato growing season in the Hawaiian Islands, namely, galling of tubers is more severe when the soil is extremely wet than in years of normal precipitation. The difficulty experienced in separating an enlarged lenticel and a nematode gall, on tubers grown in wet soil, raises an interesting question. Were all of the "galls" on the tubers in the 1937–38 potato crop of the Hawaiian Islands actually due to attacks by *H. marioni*, or were some, possibly a majority, merely enlarged lenticels? Regardless of their true nature, the tubers were of unsatisfactory appearance and growers were penalized and lost money thereby.

SUMMARY

Potatoes grown in Hawaii in 1937–38 were galled severely by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, in a growing season distinguished from others because of excessive rainfall which kept the soil wet for much of the growing season, December to April.

Potatoes were grown experimentally in nematode-infested and in chloro-picrin-fumigated soil of varying moisture content, wet soil in which the moisture probably fluctuated between 31 and 36 per cent, in dry soil with moisture content of 24 to 27 per cent, and in a soil of intermediate moisture content, 27 to 32 per cent. No claim is made that the moisture content was ever held constant, but it was controlled within certain limits.

The higher the soil moisture, the higher the tuber yields in fumigated as well as in infested soil, but the higher the soil moisture the greater the injury to tubers produced in an infested soil through galling caused by the parasite *Heterodera marioni* (Cornu) Goodey. In the presence of the nematode and in a dry soil, yields were lower than commercially profitable, therefore the soil moisture should be adequate if the root-knot nematode is present. Nematode damage to roots seemed to be greater in dry soil than in wet soil.

Evidence was found that the root-knot nematode can enter potato tubers through enlarged lenticels, which are commonly observed on tubers grown in wet soil. This is believed to be the first time this type of infection has been found. No opportunity presented itself to study the possible entry of *Heterodera marioni* into tubers bearing normal lenticels.

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INTERRELATION OF BEAN VIRUS 1 AND BEAN VIRUS 2 AS SHOWN BY CROSS-PROTECTION TESTS

RAYMOND G. GROGAN AND J. C. WALKER

Bean virus 1 and *bean virus 2,* the incitants of common mosaic and yellow mosaic, respectively, of bean (*Phaseolus vulgaris* L.) have physical properties which are practically identical (8). They differ primarily in host range, symptoms, and seed transmission. *Bean virus 1* does not infect ordinarily a considerable list of varieties of *Phaseolus vulgaris* while some strains of *bean virus 2* infect all varieties tested so far. *Bean virus 1* affects a narrower range of other legumes than does *bean virus 2.* In general the symptoms produced by *bean virus 2* are different and often more severe than those produced by *bean virus 1.* *Bean virus 1* is transmitted in bean seed. *Bean virus 2* is not reported, so far, to be seed-borne.

Inasmuch as the differences cited above are no greater than those now commonly recognized between strains of a single virus, the question arose as to whether these viruses should be regarded as distinct or as strains of a single virus. This question becomes the more pertinent since more than one strain of each virus has been observed. The present investigation was concerned with the cross-protection effect of one virus when inoculated in the bean plant in advance of the other.

MATERIALS AND METHODS

Stock cultures of *bean virus 1* were maintained in Stringless Green Refugee plants grown from virus-free seed in the greenhouse. *Bean virus 2* was maintained in plants of Sensation Refugee 1066 also grown from seed in the greenhouse. Use of the latter variety practically eliminated the possibility of a mixture with *bean virus 1* since it is highly resistant to the latter virus. The greenhouses in which studies were conducted were kept free of insects by frequent fumigation and spraying. The experimental plants were grown at temperatures usually ranging from 24° to 28° C. In all instances, unless otherwise stated, inoculations were made by rubbing with carborundum as an abrasive.

Bean virus 1 was obtained from infected seed material supplied by Dr. W. J. Zaumeyer. This virus caused leaf curling and mottle on susceptible varieties and occasionally caused the production of hydrotic or greasy pods. These symptoms agree with those reported by other workers for this virus (2, 4). On varieties possessing resistance derived from Corbett Refugee (1, 7, 9, 10, 12), it caused systemic necrosis on young plants and symptoms of black root on plants in the pod stage (5, 6). The culture of *bean virus 2* used was secured from naturally infected plants in the vicinity of Madison, Wisconsin, and is believed to be similar to the strain used by Pierce (8). It was infectious to all varieties of bean tested.

Sensation Refugee 1066 and Idaho Refugee seed were supplied by Dr. M. E. Anderson of the Rogers Bros. Seed Co. Stringless Green Refugee seed was grown at Madison in isolated plots which were carefully rogued for common bean mosaic.

Plants of Stringless Green Refugee, which is susceptible to both *bean viruses 1* and *2,* were used as test plants. The methods of conducting the cross-protection tests and methods of assay for the presence of the two viruses are described in connection with the presentation of the experimental results.

<center>EXPERIMENTAL RESULTS</center>

Specific Protection Against Bean Virus 2 Produced by Bean Virus 1

Cross-protection tests involving *bean virus 1* and *bean virus 2* were set up as follows. Each virus was inoculated to 24 Stringless Green Refugee bean plants. Nine days later when these plants had developed systemic symptoms, the *bean-virus-1*-infected plants were inoculated with *bean virus 2* and the *bean-virus-2*-infected plants were inoculated with *bean virus 1*. At the time of the second inoculation 10 healthy plants of the same age were inoculated (to serve as controls) with *bean virus 1* and 10 with *bean virus 2*. Ten days after the second inoculation practically all plants of the control groups showed symptoms characteristic of common and yellow mosaic, respectively. Test plants which were first inoculated with *bean virus 1* and later with *bean virus 2* were evidently infected with the first virus only, since they showed none of the symptoms usually caused by *bean virus 2*. However, some test plants which received the reverse treatment (*bean virus 2* followed later by *bean virus 1*) had apparently become infected with *bean virus 1* as evidenced by rolling and cupping of the young leaves, a characteristic symptom of common mosaic. To determine the presence or absence of the two viruses in the test plants, leaves from each group of plants were ground separately in a sterile mortar and inoculations were made to four plants each of Stringless Green Refugee and Sensation Refugee 1066. Both viruses could infect the former, but Sensation Refugee is highly resistant to *bean virus 1* and therefore only *bean virus 2* would develop in plants of this variety. All Stringless Green Refugee plants inoculated with juice from the group inoculated with *bean virus 1* alone, or with this virus first and *bean virus 2* later, became infected with *bean virus 1*. Sensation Refugee plants inoculated with a part of the same juice remained symptomless except for an occasional plant which developed systemic necrosis caused by *bean virus 1* (3). Stringless Green Refugee assay plants inoculated with juice from test plants inoculated with *bean virus 2* first and *bean virus 1* later became infected with *bean virus 1* in 13 of 24 cases. All Sensation Refugee plants inoculated with a portion of the same juice became infected with *bean virus 2*. This experiment was repeated three times with essentially the same results.

Seed of Stringless Green Refugee was saved from plants of the group inoculated first with *bean virus 2* and later with *bean virus 1,* and from con-

trol plants inoculated with *bean virus 1* alone. This seed was planted in the greenhouse and observed for the occurrence of common mosaic in an attempt to determine the effect of *bean virus 2* on the seed transmission of *bean virus 1*. Results (Table 1) show that when *bean virus 2* became established in a plant it reduced the amount of seed transmission of *bean virus 1*. However, since the number of seed used was small these results were considered as indicative but not conclusive.

In an experiment conducted in the same way as the one just described, except that the second inoculations were made 6 days instead of 9 days after the first, practically no cross-protection occurred. This raised the question as to how long each virus must be present in the plants before one protected completely against the other. An experiment was set up in which the second inoculations were made 6, 8, 10, 12, and 14 days, respectively, after the first. Eight plants were used for each inoculation interval and for each virus the same number of healthy plants of the same age were inoculated to serve as controls. An assay was made as described in the previous experi-

TABLE 1.—*The effect of bean virus 2 on seed transmission of bean virus 1*

History of seed plants	Seed Planted	Total Plants	Plants infected with *bean virus 1*	
			Number	Percentage
	No.	*No.*		
Inoculated with *bean virus 2* followed by *bean virus 1*	19	16	3	19
Inoculated only with *bean virus 1*	36	20	12	60

ment. When plants inoculated with *bean virus 1* were inoculated with *bean virus 2* 6 and 8 days later, very little protection occurred, but when inoculations were made at 10, 12, and 14 days no development of yellow mosaic occurred. *Bean virus 2* protected against *bean virus 1* completely at 12 and 14 days. *Bean virus 2*, therefore, required a slightly longer period than did *bean virus 1* to protect against the opposite virus.

As reported elsewhere (3), when plants of varieties in which resistance to *bean virus 1* had been derived from Corbett Refugee were inoculated with *bean virus 1* through approach grafts to Stringless Green Refugee, they showed necrosis and death characteristic of black root. Since *bean virus 2* protected against *bean virus 1* in other tests, it was of interest to determine whether it could protect against necrosis caused by *bean virus 1* if the Stringless Green Refugee portions of the grafts were inoculated with *bean virus 2* before they were inoculated with *bean virus 1*. Approach grafts were made between healthy Stringless Green Refugee and Idaho Refugee plants. Thirteen grafts were inoculated with *bean virus 2* and 13 were left noninoculated. Nine days later, when yellow mosaic symptoms had developed in both parts of the 13 grafts inoculated with *bean virus 2*, they were inoculated with *bean virus 1*. The 13 grafts which were not inoculated with *bean*

virus 2 were treated as follows: seven were inoculated with *bean virus 1*, three with *bean virus 2*, and three were left noninoculated. Results of these inoculations are presented in table 2. The Idaho Refugee portion of five grafts inoculated with *bean virus 1* alone died within a short time after inoculation. Twelve of 13 grafts which had been inoculated with *bean virus 2* before being inoculated with *bean virus 1* developed a mottle typical of yellow bean mosaic but did not become necrotic, and at maturity no root necrosis was evident.

TABLE 2.—*The prevention of systemic necrosis caused by bean virus 1 in Idaho Refugee plants by earlier inoculations with bean virus 2*

Treatment	Total grafts	Grafts which became necrotic	
	No.	No.	Percentage
Inoculated with *bean virus 2* followed by *bean virus 1* ten days later	13	1	7.7
Inoculated with *bean virus 1* at time of second inoculation above	7	5	71.4
Inoculated with *bean virus 2* at same time as above .	3	0	0.0
Noninoculated	3	0	0.0

DISCUSSION

Most studies of cross protection are based on local-lesion-mottle tests. However, such a reaction is not necessary for a successful immunity test. Pound and Walker (11) state "where two related viruses' have a differential reaction on any host their ability to immunize plants against each other can be tested in any plant in which they develop systemically." The correctness of this statement is borne out by this study with *bean viruses 1* and *2* which do not develop local lesions on any host in so far as known, but which lend themselves readily to tests for specific immunity because of their differential reactions on certain bean varieties. It was assumed that if one of the viruses by earlier inoculation could prevent the development of the second virus to such an extent that it could not be recovered by inoculation back to a susceptible variety, that specific immunity had developed. On this basis, it has been shown in this study that specific immunity was developed by *bean virus 1* to *bean virus 2* and vice versa. Plants of Idaho Refugee infected with *bean virus 2* and inoculated with *bean virus 1* by approach graft with Stringless Green Refugee became necrotic in one out of 13 cases while in plants not previously infected with *bean virus 2* five out of seven became necrotic when infected with *bean virus 1*. Limited evidence was presented which indicates that *bean virus 2* caused a reduction in the amount of seed transmission of *bean virus 1*.

Previous workers have shown that *bean virus 1* and *bean virus 2* do not differ appreciably in dilution end point, thermal inactivation, and longevity

in vitro. The only points of difference are in symptoms produced, seed transmission, and host range. The fact that they effectively cross-protect against each other in bean is further evidence that they are closely related viruses.

SUMMARY

By the use of the differential reaction to *bean virus 1* and *bean virus 2* in Stringless Green Refugee and Sensation Refugee 1066 bean varieties, the former virus was shown to effectively immunize beans against infection with the latter and likewise *bean virus 2* was shown to partially or entirely immunize beans against *bean virus 1*. *Bean virus 2* also reduced the amount of seed transmission of *bean virus 1* and reduced the amount of systemic necrosis caused by *bean virus 1* on certain resistant varieties. In view of the facts that they are very similar in dilution end point, thermal inactivation, longevity in vitro and that they protect against one another in bean, they are regarded as closely related viruses. The chief differences are in host range and in the fact that *bean virus 1* is transmitted in bean seed while *bean virus 2* is not.

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THE GENERIC AND SPECIFIC CHARACTERS OF PHYTOPATHO-GENIC SPECIES OF PSEUDOMONAS AND XANTHOMONAS

WALTER H. BURKHOLDER[1] AND MORTIMER P. STARR[2]

Many of the descriptions of the bacterial plant pathogens, especially of those forms occurring in the genera now known as *Pseudomonas* and *Xanthomonas,* have appeared identical except for the characteristic of pathogenicity. Consequently, it has been impossible to identify these bacteria successfully by means of the usual procedures of the bacteriological laboratory, and the question has arisen whether or not there are numerous synonyms present. The differences in pathogenicity possibly preclude this supposition and, where careful comparisons have been made, relatively few synonyms have been shown to exist among the phytopathogenic bacteria. One might argue that there are a limited number of true species among the phytopathogenic bacteria in these genera, and that a great many *formae speciales* exist, if one prefers to use this mycological term. This is probably not the entire story either, and, in order to investigate this question, we have taken, as our working hypothesis, the belief that where there is a true difference in pathogenic ability, some other type of difference should be demonstrable—be it cultural, biochemical, metabolic, serological, or of some other category. We do not infer that such differences necessarily explain the differences in pathogenicity.

·One of the reasons for the many identical descriptions in these genera is the failure of the investigator to recognize which bacteriological characters are generic, and which are specific. Only too frequently, the descriptions of many bacterial plant pathogens offer only generic characters. We are referring to the general run of descriptions that on the surface appear adequate, not to the large list of named plant pathogens that has been placed in appendices of Bergey's "Manual of Determinative Bacteriology," because the described characters of these are too few and too confused for one to be certain of the species. In the present paper, the writers are endeavoring to point out what characters are generic, and what characters might be used in separating the phytopathogenic species for the genera *Pseudomonas* and *Xanthomonas.*

In making this report, we are not proposing a final scheme and key to the species, nor do we consider the minutiae of our results absolutely conclusive since there was not available, in all cases, a sufficient number of isolates to assure statistical validity. However, the results do indicate means of distinguishing some of the bacteria, and, what is perhaps more important, point the way toward the likelihood that further study will uncover additional means of separation.

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METHODS

The cultures which were studied represent a collection which was assembled over the years for a variety of purposes (20, 22, 23). In the majority of cases, they are cultures which the writers isolated and proved pathogenic at the time of isolation. In some instances, the bacteria were received from other investigators, who were working with those species, and whose identifications we had reason to consider authentic.

The search for specific differences at times involves the trial of techniques and criteria which are not at present in routine use for this group of bacteria. The experimental methods used in the present investigation to separate species of phytopathogenic bacteria are now given.

Tolerance of sodium chloride. Salt-tolerance of phytopathogenic bacteria has received comparatively little systematic study. In some cases, the NaCl-tolerance of individual species is included in the descriptions, but the conditions under which this characteristic was determined varied with the laboratory to an extent which prohibits comparison of the results given by different workers. In the present study, the following procedure was used. A series of salt broths was prepared using as a base 0 3 per cent of Bacto yeast extract and 0.5 per cent Bacto tryptose in distilled water, with the NaCl concentration ranging from 1 to 10 per cent in one per cent steps. The reaction after autoclaving was pH 7.0 to brom thymol blue and the media were used before evaporation took place. In order to avoid variations in salt-tolerance which occur as a function of the age of the bacterial cells being tested (2, 14) uniform loopsful of 48-hour-old cultures in yeast extract-tryptose broth without added NaCl were used as inoculum. The turbidity that developed in the several salt concentrations, and in the control medium without added salt, was examined visually every day for a week and, in the case of a few slow-growing organisms, for two weeks.

Tyrosinase activity. Some phytopathogenic bacteria color the ordinary peptone media.brown. Certain species are especially active in this respect (3, 4, 5, 15, 16, 17) and the suggestion has been made that the brown coloration be used as a taxonomic character or as an aid in isolating actively browning bacterial species from infected plants. Other than such incidental observations, little attention has been devoted to the problem; nor have adequate explanations been offered of the mechanisms whereby phytopathogenic bacteria produce these colorations. Indeed, there is a tendency to assume, without direct experimental evidence, that any browning of culture media is a "tyrosinase reaction"—actually an oxidation and polymerization of the amino acid tyrosine to dark melanin pigments.

Tyrosinase tests of *Pseudomonas* isolates were made in a liquid asparagin-glycerol-tyrosine medium prepared by omitting agar from the formula given by Starr (19). The *Xanthomonas* species were tested in liquid media prepared by adding 0.05 per cent tyrosine to a solution (20) which satisfies the minimal nutritive requirements of each species. The tyrosine medium and a tyrosine-free control medium were inoculated from young agar slant

cultures and incubated at 28° C. Cultures were observed periodically for growth and for pigmentations of the medium. A culture was considered tyrosinase-positive if it grew in both media, and produced, only in the tyrosine medium, a red color which eventually became dark brown.

Some of the cultures were tested, also, on an agar tyrosine medium (19). In general, the cultures which browned the liquid medium also browned the agar medium. In addition, a number of strains which appeared tyrosinase-negative in the liquid medium were able to make distinct red-to-brown colorations of the agar medium. Inasmuch as these differences cannot at this time be explained, and since the results in liquid media show promise of utility in determinative bacteriology, the data obtained from experiments in solid media will not now be presented in full or discussed further.

Liquefaction of sodium ammonium pectate. The ability of phytopathogenic bacteria to decompose pectic substances has never been systematically studied. The scattered literature reports experiments with a variety of ill-defined "pectin" preparations, and it is likely that some of the workers in this field had not realized the heterogeneous nature of pectic substances or of pectic enzymes (12, 13). Following Jones' pioneer study (11), the soft-rotting of fresh tubers and fleshy roots has been considered by many investigators as synonymous with pectinolytic activity, although this supposition is not always proved. One of the reasons for the lack of knowledge in this field has been the unavailability of pectic substances which lend themselves to routine microbiological use. A preparation of sodium ammonium pectate has recently become available,[3] which, with the addition of a calcium salt, can be made into media similar to gelatin and can be sterilized by autoclaving. The properties and use of this material are given by Baier and Manchester (1) and by Starr (21). We have prepared media of this pectate, with and without the addition of peptone or yeast extract, and have cultured phytopathogenic bacteria in these media. If the pectate is utilized, a liquefaction takes place. We do not claim, however, that organisms that liquefy this medium are necessarily able to attack the pectic substances of the middle lamella of all plant cells.

Utilization of sodium tartrate. The utilization of sodium tartrate as the sole carbon source had been shown, in preliminary experiments, to be of some differential value for the phytopathogenic species of *Pseudomonas.* Observations on a more extensive series of bacteria are now reported. The sodium tartrate was supplied at a concentration of 0.15 per cent in the synthetic medium of the "Manual of Methods for Pure Culture Study of Bacteria" (7). This medium was seeded heavily, incubated, and examined for growth and alkali production at intervals up to 2 weeks. These experiments are reported with the realization that possible growth factor deficiencies and toxicity of the tartrate were not controlled, and that "failure to grow" may actually result from one of these factors rather than from unsuitability of tartrate as carbon source. On the other hand, as will be seen

below, the medium, when used as specified herein, does give results of differential value.

Reduction of nitrates. The reduction of nitrates to nitrites has been used extensively in the past with varying results. Before attempting to employ this reaction one should read Conn's suggestions (8) and avoid the many pitfalls he lists. In the present investigation, the bacteria were grown from large inocula in the synthetic nitrate medium described in the "Manual of Methods for Pure Culture Study of Bacteria" (7). Tests were made at the end of 2 days and at various times up to 2 weeks, for both nitrites and nitrates using the reagents recommended in the manual. The experiment was repeated three times. The species also were grown in beef extract-peptone medium plus KNO_3 and tested for nitrites. This latter medium is not so reliable as the synthetic medium because in many cases where a reduction was readily demonstrated in the synthetic medium it was not demonstrated in the beef extract-peptone broth. The latter medium, however, supported better growth. One series of tests also was made for ammonia in the synthetic solution where the *Pseudomonas* species were being grown, but the only positive reaction obtained was with *P. caryophylli.*

PSEUDOMONAS

The genus. The phytopathogenic members of *Pseudomonas* have been characterized in Bergey's "Manual of Determinative Bacteriology," but a description of this group is desirable at this point in order to indicate the generic characteristics of the plant pathogens. A member of this genus is a medium-sized rod, Gram-negative, with 1–6 polar flagella, and without spores. It turns milk alkaline; produces no indol nor H_2S; utilizes monosaccharides but not as a rule the higher carbohydrates with the exception of sucrose. Starch is not hydrolyzed. Most species can utilize natural asparagine as the sole carbon and nitrogen source. These bacteria are aerobic, or facultatively anaerobic with optimum temperatures at 27°–30° C. The species remain viable on beef extract–peptone agar for many months but not on potato-dextrose agar.

The species. What characters can be used to separate the species in the genus *Pseudomonas?* At present, not many have been studied, but those which are known should be stressed, and tests to determine them in all instances should be carefully conducted. Clara (6) pointed out that the plant pathogens in the genus *Pseudomonas* could be separated into two groups; those that can utilize sucrose and those that cannot. The character appears to be a reliable one and, since many of the species of *Pseudomonas* grow readily in a simple synthetic solution, it can be determined easily. Where a growth factor is required it may be added, but unless purified chemicals and glassware are used it will probably not be necessary. Filtered sugars are recommended.

Gelatin liquefaction is a second specific character of importance on which the genus may be subdivided and is one usually recognized. It is easily determined, and as a rule is used in all descriptions.

The ability to reduce nitrates to nitrites, ammonia or nitrogen, while it had not been neglected with these pathogens, had rarely been very carefully determined. It is thought by certain investigators that all the *Pseudomonas* species reduce nitrates, but our work does not entirely substantiate this belief. However, some of the pathogens that have been described as non-reducers have been shown in this investigation actually to possess this ability.

The following species were found by us to be able to reduce nitrates to nitrites: *Pseudomonas aceris, P. alliicola* [5],[4] *P. angulata* [3], *P. apii* [2], *P. aptata, P. atrofaciens* [2], *P. barkeri, P. berberidis* [2], *P. caryophylli* [2], *P. delphinii* [2], *P. glycinea* [3], *P. lachrymans, P. lapsa, P. mori* [3], *P. papulans, P. savastanoi* var. *fraxini, P. syringae* [11], *P. tabaci* [9], *P. tomato* [3], *P. tonelliana, P. viridilivida, P. washingtonia.*

Pseudomonas striafaciens and *P. viburni* [2] gave very weak nitrite reactions, but no nitrites were detected in the tubes of any of the remaining cultures which were used for this and most of the other experiments described herein: *Pseudomonas cichorii, P. coronafaciens* [2], *P. maculicola, P. marginata* [2], *P. mellea, P. phaseolicola* [5], *P. pisi* [2], *P. primulae, P. ribicola* [3], *P. ribes* [2], *P. sesami* [2], and *P. woodsii* [2].

Of the phytopathogenic pseudomonads tested, there was growth in the tartrate medium only of *Pseudomonas aceris, P. alliicola, P. aptata, P. barkeri, P. caryophylli, P. cichori, P. marginata, P. mellea, P. pisi, P. tabaci, P. viridilivida,* and *P. washingtonia.*

In the tyrosine medium, about half of the tested phytopathogenic *Pseudomonas* species produces a definite brown pigment, which more often turns an olive-green when it combines with the water-soluble green pigment so frequently produced by members of this group. These tyrosinase-positive species are: *P. alliicola, P. angulata, P. apii, P. barkeri, P. berberidis, P. coronafaciens, P. delphinii, P. maculicola, P. papulans, P. primulae, P. ribicola, P. savastanoi* var. *fraxini, P. tabaci,* and *P. viridiflava.* Four species, *Pseudomonas pisi, P. savastanoi, P. sesami,* and *P. tomato,* also produced this coloration but to a lesser extent. The other pseudomonads failed to produce the red-to-brown coloration under the conditions specified in the section on "Methods"; although many of these cultures made brown pigment from tyrosine under other testing conditions (18). It is noteworthy that *P. caryophylli* produces its typical brown cell-pigment regardless of the addition of tyrosine to the medium.

The common saprophyte of this genus, *Pseudomonas fluorescens,* is usually reported as lipolytic, but most of the phytopathogens are "not lipolytic" by the spirit blue agar method.[5] The reaction of some of these has been determined (22), and aids in classifying these bacteria.

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4 Numerals in brackets indicate the number of isolates of a particular species which was used by us, when more than one was available.

5 "Lipolytic" is used here in the limited sense in which it was employed by Starr and Burkholder (22); that is, on the basis of the blue reaction shown in the spirit blue–cottonseed oil–agar method. A "lipolytic" reaction on this medium probably indicates hydrolysis of the cottonseed oil, with liberation and accumulation of certain fatty acids that cause the spirit blue indicator to become blue. Some fat-hydrolyzing, oxidative

The *Pseudomonas* species which were tested do not liquefy the pectate medium. This is especially interesting in the case of *P. alliicola,* that causes a soft-rot of onion. Presumably this species possesses other pectic enzymes than the one which attacks the pectate of this medium, or it is able to induce the rot by some other means.

The NaCl-tolerance of phytopathogenic pseudomonads may, in some cases, be used as a determinative character, although generally the differences between species are not great. Some species (e.g., *P. berberidis, P. solanacearum, P. viburni,* and *P. woodsii*) are characteristically inhibited by as little as 2 per cent NaCl. On the other hand, two cultures, one each of *P. marginata* and *P. viridilivida,* required 10 per cent salt to completely inhibit growth; these data, however, should be checked with additional isolates.

There are but few other properties that can be used as determinative characters in this group. Milk, as stated above, always turns alkaline, but certain species clear it, whereas others do not. The production of a water-soluble green pigment in certain media is a fairly common, but by no means universal, property. Non-pigment-forming strains have been reported in species ordinarily forming the pigment. Like all identifications based on pigmentation, there are limitations to its use.

<div align="center">XANTHOMONAS</div>

The genus. Members of the genus *Xanthomonas* (9) are readily recognized by their abundant, slimy, yellow growth on sugar-containing media. The yellow pigment is a water-insoluble material having the properties of a carotenoid and a maximum absorption in ethanol at about 445 mμ (19). All xanthomonads are aerobic, gram-negative, non-spore-forming, small rods, motile with a single polar flagellum. They are proteolytic, liquefying gelatin and peptonizing milk with, as a rule, the deposition of tyrosine crystals in the latter. They form hydrogen sulfide, but not indol; most of them give the "lipolytic" reaction[5] on spirit blue-cottonseed oil agar (22); they form acid, but not gas, from a variety of carbohydrates; their minimal nutritive requirements are relatively simple (20), yet they are unable to use asparagine as the sole source of both carbon and nitrogen (23); they are not particularly tolerant of sodium chloride, the growth of many of them being retarded by only 2 or 3 per cent of NaCl, and all of them fail to grow in a 5 per cent NaCl medium; they remain viable on potato-dextrose agar or on yeast extract-dextrose-CaCO$_3$ agar, but die off early when cultured on sugar-free beef-peptone or on yeast extract agar media.

The species. Unequivocal identification, by laboratory procedures, of all *Xanthomonas* species is as yet an impossibility. From published descrip-

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bacteria may so completely decompose the cottonseed oil, that the fatty acids are degraded beyond the point where they can effect a permanent color change of the indicator. Such a thorough decomposition of cottonseed oil would, perhaps, not be recognized by the spirit blue–agar technique, and fat-splitting organisms capable of this type of decomposition might be classed as not "lipolytic" by the spirit blue–agar procedure.

tions, many of the named species seem identical in cultural characteristics and appear to differ only in that they had been isolated from some particular host. Since the cross-inoculation tests reported in the original descriptions of many of these species have been narrow in scope or entirely lacking and later tests are scattered through the literature, the idea is prevalent that a great number of synonyms exist in this genus. Probably, there is some synonymy; however, at least one fairly comprehensive series of cross-inoculation experiments has been carried out (24, 25), wherein 17 species and varieties of *Xanthomonas* were shown to be completely specific in their pathogenicity.

Let us see in how far bacteriological tests help distinguish *Xanthomonas* isolates which originate from different hosts. The ability to hydrolyze starch divides the genus into two groups. A few of the species are not lipolytic[5] on spirit blue-cottonseed oil agar. A recent study of the nutrition of this genus (20) shows that at least three species have distinctive nutritive requirements which, after more study and when used with suitable precautions, might be employed as determinative characters.

Some exceptional xanthomonads reduce nitrates to nitrites; in our own experience[6] nitrate-reduction to nitrite was observed for the 2 isolates of *Xanthomonas barbareae*, for 2 of the 4 tested *X. begoniae*, for the single cultures of *X. cucurbitae* and *X. lactucae-scariolae*, for the 2 *X. papavericola*, and for about half of the *X. geranii* and *X. pelargonii* isolates. In addition, 2 species which we do not believe belong in this genus, *X. beticola* and *X. rubrilineans*, are strong nitrate-reducers. Because of the existence in some species of strains differing in their reactions to this test, it should be used with caution for determinative purposes. Furthermore, as noted above we do not consider all our results necessarily valid for taxonomic purposes because of the limited number of isolates of some species. We do wish to indicate that only a minority of *Xanthomonas* species are able to reduce nitrates to nitrites, and that strain differences are sometimes seen.

Our trials with the pectate medium show that about a half of the tested *Xanthomonas* species was able to liquify this medium; viz., *X. barbareae, X. campestris, X. carotae, X. geranii, X. incanae, X. lespedezae, X. manihotis, X. papavericola, X. pelargonii, X. phaseoli* var. *sojensis, X. taraxaci* (weak or doubtful), *X. vascularum, X. vesicatoria* from tomato, *X. vesicatoria* var. *raphani,* and *X. vignicola.* Generally, the pectate was liquified more slowly by the xanthomonads than by soft-rot *Erwinia* species. The ability to liquefy pectate was not correlated in any apparent manner with a capacity to digest starch, nor with any particular pattern of disease symptomatology.

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[6] The following *Xanthomonas* cultures were used in most of our investigations (the figure in brackets indicates the number of isolates generally used, when more than one was tested): *Xanthomonas barbareae* [2]; *X. begoniae* [2]; *X. beticola* [2]; *X. campestris* [5]; *X. campestris* var. *armoraciae; X. carotae; X. corylina* [3]; *X. cucurbitae; X. geranii* [3]; *X. hederae; X. incanae; X. juglandis* [4]; *X. lespedezae* [2]; *X. malvacearum* [2]; *X. manihotis* [7]; *X. papavericola* [2]; *X. pelargonii* [2]; *X. phaseoli* var. *fuscans* [3]; *X. phaseoli* var. *sojensis* [4]; *X. pruni* [4]; *X. rubrilineans; X. taraxaci; X. translucens* including 5 *formae speciales* [8]; *X. vascularum; X. vesicatoria* [4] from tomato and [6] from pepper; *X. vesicatoria* var. *raphani; X. vignicola* [2]; and *X. vitians.*

Only three of the *Xanthomonas* species which we tested are characteristically tyrosinase-positive, producing in tyrosine-containing media red colors which soon become brown. *X. phaseoli* var. *fuscans* is most active in this respect; *X. geranii* and *X. pelargonii* also give this reaction, whereas the other *Xanthomonas* species are incapable of producing the red-to-brown coloration under the conditions specified in the section on "Methods." However, some additional cultures do produce red-to-brown reactions under other conditions (18).

The tolerance of the genus *Xanthomonas* for sodium chloride is not great. As noted above, no xanthomonad grows in 5 per cent NaCl broth.[7] Several species grow in 4 per cent salt broth; many grow in 3 per cent. *X. pruni* is probably the most sensitive species, the growth being retarded by 1 per cent NaCl and inhibited completely by 2 per cent. This characteristic seems stable enough for determinative purposes; indeed, the uniformity of results for the replicate isolates of a given species was rather surprising in view of the diverse origins of the cultures.

Another promising approach to this problem is provided by the recent work of Elrod and Braun (10) with agglutination reactions, using antigens low in slime concentration. They were able to group *Xanthomonas* species into several categories, thus aiding identification on some subgeneric level.

SUMMARY

The majority of phytopathogenic bacteria are now grouped in the genera *Pseudomonas* and *Xanthomonas*. The descriptions of many of the species, unfortunately, have offered mainly characteristics of use only in identifying the genus to which they belong; consequently it is often impossible to key out, by means of the usual bacteriological procedures, the species of these two genera. In the present paper, we have endeavored to point out those cultural and biochemical properties that are useful in characterizing the genera of phytopathogenic *Pseudomonas* and *Xanthomonas*. For determining the species, there are presented reactions that have proved useful in the past, as well as experimental work on the utilization of pectate and tartrate, production of a brown pigment from tyrosine, and the ability to grow in the presence of various concentrations of sodium chloride.

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          AND
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[7] Incidentally, this characteristic affords another method for distinguishing the corn-wilt bacterium, *Bacterium stewartii*, from xanthomonads; the corn-wilt organism generally grows in the presence of 6 or 7 per cent of salt, at which level no *Xanthomonas* species develops.

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PHYTOPATHOLOGICAL NOTES

Pellicularia Target Spot Leaf Disease of Kenaf and Roselle.[1]—Kenaf, *Hibiscus cannabinus* L., was introduced to the Tingo María zone of Peru in 1943 as one of the first fiber plants brought in for adaptability trial by the Estación Experimental Agrícola de Tingo María (now named Estación Central de Colonización en Tingo María). The 1943 planting and subsequent plantings during 1944 and 1945 were small and were made primarily for obtaining seed to maintain the selection. In 1946 larger scale trials were started in response to the need for a domestic grown fiber for bags. The 1946 plantings were successful and in general disease-free. The success of these plantings resulted in an area increase in 1947 to allow sufficient production of fiber for a commercial test of its uses in Peru. No disease problems were apparent until just before harvest. At that time small, occasionally zonate leaf spots characterized by a red halo were observed. Within a few weeks the infestation was general through the planting and during the closing weeks of the wet season (April) the large zonate spots (Fig. 1, A, and B) were present on almost every leaf. On the upper side of the leaf (Fig. 1, A) the spots have the typical zonate formation bordered by a brilliant red halo. On the under side of the leaf the zonate character is less distinct and the red halo almost completely lacking (Fig. 1, B). Characteristically a cobwebby growth of mycelium is found 5–10 mm. in advance of the necrotic zonate areas. Observations indicate that infections occur primarily on young leaves and develop as the leaves grow.

Tissue isolations from advancing margins of the leaf spots uniformly yield a species of *Rhizoctonia*. On the older portions of the spots, especially against the veins, fruiting of a species of *Pellicularia* may occasionally be found. This species of *Pellicularia* has spores averaging 3.4–4.3×6.8–8.5μ and under present concepts of this genus is referable to *P. filamentosa* (Pat.) Rogers.[2]

The first introductions of roselle, *Hibiscus sabdariffa* L., were made in 1947 about the time the disease had become widespread on the kenaf. The roselle was not planted near the kenaf but was in close proximity to nursery beds of rubber heavily infected with a similar leaf spot also caused by a strain of *Pellicularia filamentosa*.[3, 4] These plants were almost at once attacked by *P. filamentosa*, which causes a leaf spot on them, and were

[2] Rogers, Donald P. The genus Pellicularia (Thelephoraceae) Farlowia 1: 95–118. 1943.

[3] Kotila, John E. Rhizoctonia foliage disease of Hevea brasiliensis. Phytopath. 35: 739–741. 1945.

[4] Lorenz, Rolland C. A new leaf disease of Hevea in Peru. Journal of Forestry (In press).

severely retarded in growth. Microscopically the Pellicularia stage appears identical with that on kenaf and is indistinguishable from the Rhizoctonia stage isolated from the leaf tissue. Symptoms of the disease on the leaves are almost identical (Fig. 1, B and C). Rhizoctonia has been reported on roselle in El Salvador[5] causing stem canker but is probably not a manifestation of this same disease. Insofar as can be determined, the diseases herein reported have not previously been described either in Peru or elsewhere on kenaf or roselle.

Okra, *H. esculentus* L., another member of the genus *Hibiscus,* is grown in close proximity to the kenaf but thus far has remained free of the disease.

In addition to the leaf disease on rubber previously mentioned, web-blight
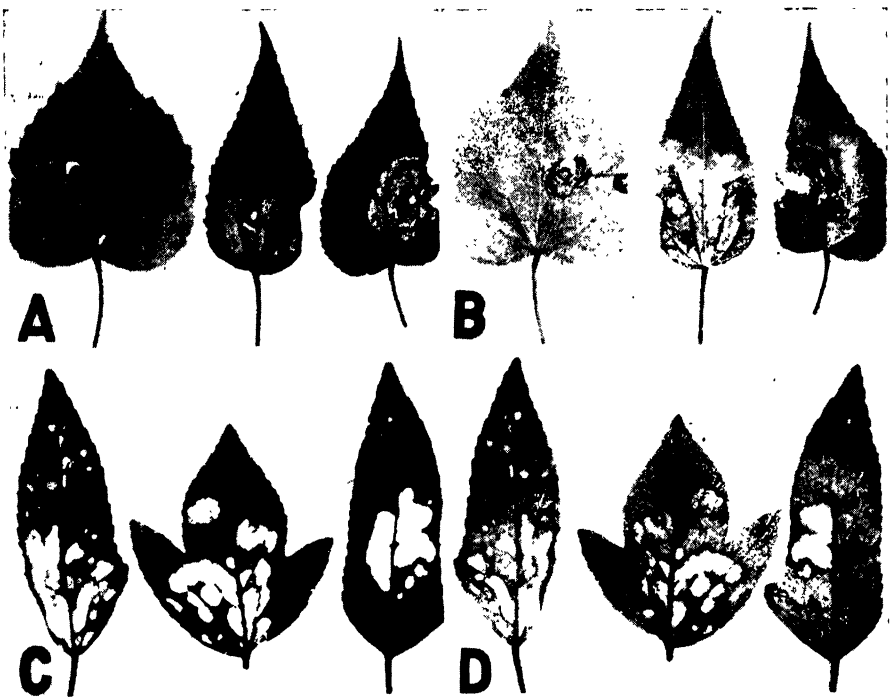
FIG. 1. A. Pellicularia target spot disease on upper surface of leaves of kenaf. B. Lower surfaces of same leaves showing cobwebby mycelial growth. C. Similar disease on upper surface of roselle leaves. D. Lower surface of same leaves showing mycelial growth.

of beans,[6] heretofore not observed in this zone, made its appearance in the experimental fields of the Station about the same time as the disease on kenaf. This disease is also caused by *Pellicularia filamentosa.*[2]

Artificial inoculation with spores has not been possible since thus far none have been produced in culture and naturally produced spores are not

[5] Reinking, O. A. Diseases of Roselle fiber plants in El Salvador. U. S. Bureau of Plant Industry, Plant Disease Reporter 29: 411–414. 1945.

[6] Weber, George F. Web-blight, a disease of beans caused by Corticium microsclerotia. Phytopath. 29: 559–575. 1939.

sufficiently abundant to allow their use. It has, however, been possible to use infected leaves and agar cultures of the *Rhizoctonia* stage for cross inoculations. In these cross inoculation trials the strain of *Pellicularia filamentosa* from beans was included since the leaf symptoms were similar, but primarily as a fungus control on the inoculation method.

Leaves of roselle and rubber with active lesions were placed in contact with young leaves of these hosts and kenaf, and a disease with similar symptoms developed on rubber and roselle but not on kenaf. In another trial, a leaf spot developed on roselle, kenaf, and rubber when infected material from rubber was used as the source of inoculum.

Agar cultures of the Rhizoctonia stages from the disease on kenaf, roselle, beans, and rubber were subsequently used to inoculate 4 leaves each by placing agar blocks of mycelium between the lower surfaces of two leaves and clamping them together. With this method the isolate from kenaf successfully produced the disease in 4 of the 4 inoculations on rubber, in 4 of the 4 inoculations on kenaf, and in 2 of the 4 inoculations on roselle. The isolate from roselle produced the disease only on 4 of the 4 leaves of roselle, the rubber isolate produced the disease only on rubber. The isolate from beans produced typical target spots of more virulent type than the natural spots in all 4 of the inoculated leaves of kenaf, roselle, and rubber. Although not entirely conclusive, these preliminary inoculations indicate that the target spot leaf diseases of rubber, kenaf, roselle, and web-blight of beans are apparently all manifestations of disease produced by the same or slightly different strains of *Pellicularia filamentosa*. Check inoculations on all hosts using sterile agar blocks resulted in no infections and no injury to the leaves.

The disease on the present kenaf crop does not appear to have seriously affected fiber production. Infected roselle plants developed much slower than healthy individuals. During the dry season the disease is still in evidence but its intensity is much reduced. Roselle and kenaf, if grown during the wet season, may need spray protection at least during the first stages of growth.—BOWEN S. CRANDALL, Pathologist, Office of Foreign Agricultural Relations, United States Department of Agriculture, and Technical Chief, Department of Plant Pathology and Entomology, Estación Central de Colonización en Tingo María, Peru.

Physalis angulata, a Test Plant for the Potato Leafroll Virus.—A study of potato leafroll was handicapped by the fact that a suitable test plant was not available which could be grown easily and which would show distinct symptoms of the disease.

The potato plant is not suitable for studying some phases of the leaf-roll problem. It is propagated vegetatively and hence each tuber used for the experiments must be checked to ascertain that it is not already harboring the virus. Potato plants are relatively large and occupy much valuable space in the greenhouse. The symptoms of leafroll often are

slow to develop or are masked, and often it is necessary to grow the progeny of the test plants the following year before the results of the inoculation studies can be obtained.

These handicaps in studying the leafroll virus were eliminated by using *Physalis angulata* L. as the test plant. This plant is propagated from true seed which readily germinate without requiring a long rest period. It is rather small and easily grown in small pots in the greenhouse and it is a good host plant for the different potato aphids. Furthermore, *the symptoms of the leafroll disease become apparent on the* the test plant about 10 days after the plants have been inoculated. This plant shows distinct symptoms of the leafroll disease when inoculated

FIG. 1. A. Left, healthy *Physalis angulata* control. Right, *P. angulata* with leafroll transmitted by *Myzus persicae* (Sulz.) from diseased Katahdin potato plant. B. Small plant covered with plastic cage after being inoculated with aphids.

with viruliferous aphids, the infected plants becoming dwarfed with chlorotic and rolled leaves (Fig. 1, A).

The plants used for the leafroll studies are grown in 4–inch pots in the greenhouse and are inoculated with aphids when from 2 to 3 inches high. The viruliferous aphids are transferred to the test plants with a camel's-hair brush. Plastic cages are fitted over the inoculated plants and the aphids are allowed to feed about 5 days (Fig. 1, B). The cages are then removed and the aphids killed by fumigation. The plants are examined for leafroll symptoms from 10 to 20 days after they are inoculated.

The use of *Physalis angulata* as a test plant has materially shortened the time necessary for making inoculation experiments with the leafroll virus and has been useful in studying certain phases of the aphid-leaf-

roll relationship. The studies indicate that although certain non-solanaceous wild plants normally serve as breeding hosts for the potato aphids, they apparently do not harbor the leafroll virus. Infested potato plants in the growers' seed stocks appear to be the chief source of infection.

The studies confirmed the data obtained with the potato, that the peach aphid, *Myzus persicae* (Sulz.), is the chief vector of potato leafroll in Maine. The potato aphid, *Macrosiphum solanifolii* (Ashm.), and the buckthorn aphid, *Aphis abbreviata* Patch, did not readily transmit the leafroll virus to *Physalis angulata*.

It was shown that leafroll can be transmitted by a single viruliferous peach aphid inoculating the Physalis plants. However, often no infection resulted when single aphids were used. About 75 per cent of the plants became infected when five viruliferous aphids were used to inoculate each Physalis plant.

The virus was transmitted by all of the nymphal stages of the peach aphid, and the younger aphids (first and second instars) transmitted the disease as readily as did the mature apterous aphids.

Winged aphids are very numerous in Maine during late summer when they are migrating from the potato fields. It was assumed that these aphids are very important factors in the dissemination of the leafroll virus. The data showed that less than 0.1 per cent of the winged migrating peach aphids were viruliferous when tested on *Physalis angulata*. About 24 per cent of the winged peach aphids were viruliferous when it was known they had fed on potato plants which had the leafroll disease.

The leafroll virus was readily transmitted from infected Physalis plants to healthy Katahdin potato plants by the peach aphid, and typical symptoms of the disease resulted.—CHARLES HOVEY, formerly of the Maine Agricultural Experiment Station, and REINER BONDE, Maine Agricultural Experiment Station, Orono, Maine.

YELLOWS AND NECROTIC RING SPOT OF SOUR CHERRIES IN ONTARIO: INOCULATION EXPERIMENTS[1]

G. H. BERKELEY AND R. S. WILLISON

In 1939 Keitt and Clayton (4) reported that the disease of sour cherries, previously known in Wisconsin as physiological yellow leaf, was graft-transmissible and therefore of virus origin. They proposed renaming the disease cherry yellows (5). Two years later, 1941, experimental evidence was obtained in Ontario, that the so-called physiological yellow leaf long prevalent in that Province was also caused by a virus and could be transmitted to Montmorency cherry and to peach by budding. Comparison of symptoms on sour cherry left little doubt that the Ontario disease was cherry yellows.

A second disorder of sour cherries, now called necrotic ring spot (1), has also been present in Ontario for many years, though not recognized until 1939. In 1940 this disease was shown to be a virosis when typical symptoms were expressed on Montmorency and a variety of Duke cherry as a result of inoculation by budding from affected trees.

Both these diseases have been found widespread in sour cherry orchards in Ontario and are often responsible for considerable reduction in yield. The present paper is a report on certain phases of the investigations of these two diseases, carried out over the last seven years.

SYMPTOMS ON MONTMORENCY

Cherry yellows. In Ontario, diagnostic foliar symptoms usually appear, about three or four weeks after petal fall, as a mottling of the leaves with chlorosis between the veins or as a more or less complete yellowing, depending on the severity of symptom expression. In general, the tissue along the larger veins retains its green color after the interveinal portions have turned yellow (Fig. 1, A). Soon after the color change, the affected leaves drop, though in some seasons it is not uncommon for green leaves also to be cast. In some cases comparatively slight casting of leaves occurs, while in others the defoliation is so severe that the ground is literally covered with leaves. Environmental factors, especially the temperatures prevailing during leaf development, as shown by Moore and Keitt (7) and Mills (6), play an important role in determining the amount of defoliation.

There is generally but one wave of defoliation of any consequence in Ontario, usually in mid-June. Some yellowing and casting of leaves may occur later in the season but this is not common and is very limited in amount and duration. Diagnosis of cherry yellows in an orchard is therefore restricted to a period of from one to two weeks after symptoms first appear.

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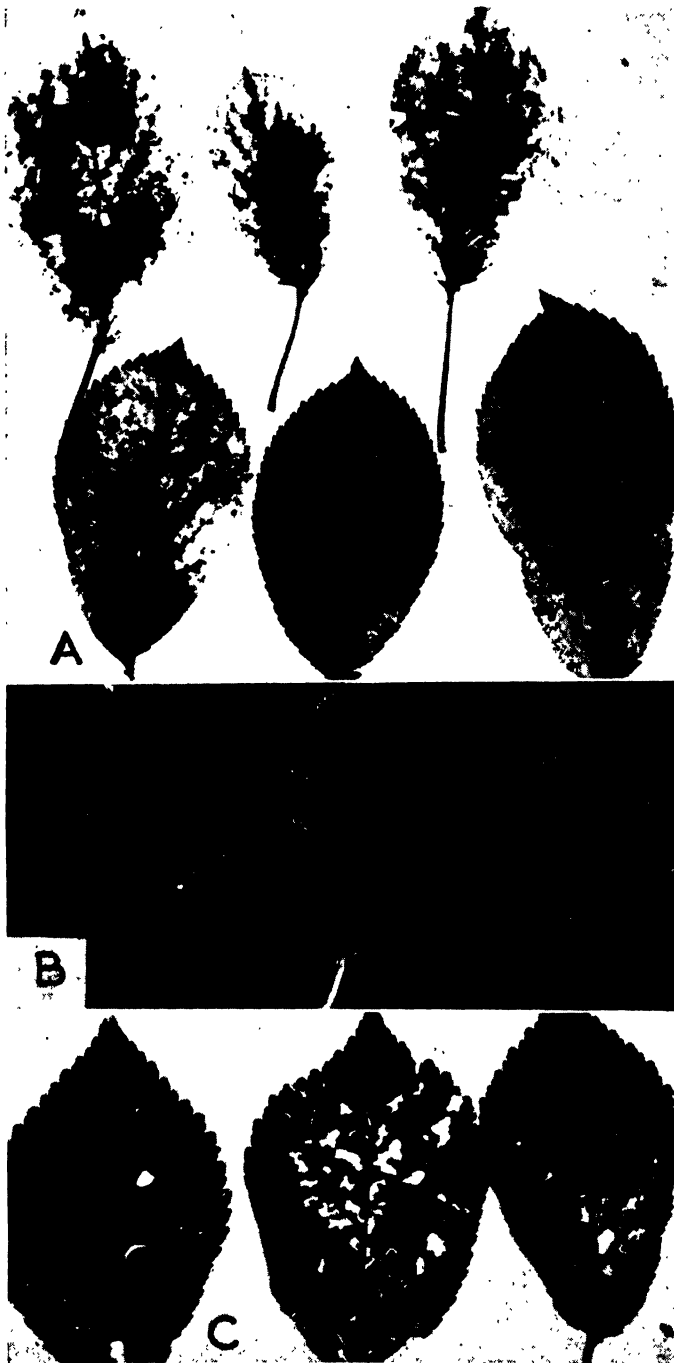
FIG. 1. A. Symptoms of cherry yellows on Montmorency, showing retention of green color around veins. B and C. Symptoms of necrotic ring spot on Montmorency showing ring spot (B) and shot hole (C) stages.

In addition to causing leaf symptoms, yellows also affects fruiting habit and growth. The spur systems of affected trees in time become greatly reduced, and with them the crop, though the fruit itself is of good quality and often larger than that on healthy trees. There is also a tendency for lateral leaf buds either to grow into shoots or, more often, to fail to develop. The ultimate effect is heavy leaf growth towards the tips of the branches and excessive lengths of barren wood back of the tips. Growth of this type causes the branches to droop, giving a "weeping" effect. This characteristic alone is not regarded as a reliable criterion of yellows infection as it also may result from bud weakening or failure from other causes.

Yellows is not a lethal disease. Trees known to have been infected with yellows for several years still produce reasonably satisfactory crops.

Necrotic ring spot. The first and most striking symptom of necrotic ring spot is delayed foliation, which may be confined to a few branches only or may be more or less general throughout the tree. That is, at the time the first leaves on healthy trees are fully expanded, those on affected trees or branches may be partially unfolded or only just emerging from the bud (Fig. 2, B). If the first unfolding leaves on affected branches are examined carefully, numerous fine translucent to brownish spots, arcs, or rings having an etched appearance are evident (Fig. 1, B). Sometimes concentric rings are present. The surface of such leaves is rough and their margins are wavy, giving the leaves a crinkled appearance. In the earliest stages, the markings are seen best in reflected light. Later, chlorotic and necrotic areas develop, the necrotic tissue falls out, leaving the leaves "shot holed" or tattered, depending on the location and amount of necrotic tissue (Fig. 1, C). Often the tip, margin, and much of the lamina become wholly or partially necrotic. The remaining tissue is normal in color, though the leaves themselves tend to be considerably smaller and rounder than normal. Severely affected leaves are frequently cast but may persist throughout the season. The etched markings, if they do not become necrotic, tend to disappear.

Symptoms similar to those on the leaves also appear on the sepals. The blossoms may be almost sessile or have more or less shortened pedicels.

It is characteristic of necrotic ring spot that diagnostic symptoms are apparent only at the beginning of the growing season. A tree severely affected in May or early June may appear to be healthy later in the same season, and, moreover, has few or no symptoms in the second and succeeding years. However, if infection was localized in a few branches the first year, other branches of the same tree may be severely affected the next spring. In affected trees, with few exceptions, the severe or shock symptoms of necrotic ring spot are not recurrent, though mildly etched rings and markings are found frequently in any season on the first formed leaves. After the initial shock symptoms have developed, there is a tendency for the foliage to become less dense, so that the tree has a more open appearance than normal.

FIG. 2. A. Symptoms on peach inoculated with cherry yellows virus; left, die back and rosette on peach seedlings; right, rosette symptoms on peach which was cut back above inserted yellows bud. B. Delayed foliation from necrotic ring spot on Montmorency (left) in comparison with healthy branch (right).

Materials and Methods

To obtain sources of infective material, several orchards were surveyed twice annually, in early spring and in mid-June, to locate cases of necrotic ring spot and cherry yellows, respectively.   At each survey, branches, from which bud sticks were to be taken later, were marked on selected trees showing symptoms of the required disease.   For obvious reasons, the selections were divided into three categories: (i) trees in which only symptoms of necrotic ring spot were observed; (ii) trees showing symptoms of yellows but not of necrotic ring spot during .the period of the surveys; and (iii) trees known to be infected with both diseases.   In most cases, the orchards had been under observation for at least two years before bud sticks were cut. All inoculations were made outdoors by the budding method.

The range of differential hosts included *Prunus persica* (L.), var. Elberta and Rochester, and seedlings; *P. domestica* (L.), var. Italian prune and Lombard plum; and *P. cerasus* (L.) var. Montmorency.   One- or two-

TABLE 1.—*Numbers of sources from which cherry yellows and necrotic ring spot viruses were transferred to the several hosts*

Host	Cherry yellows	Necrotic ring spot
Peach		
Rochester	8	4
Elberta	8	4
seedling	8	4
Italian prune	2	2
Lombard plum	4	4
Montmorency cherry	8	3

year-old nursery trees of the named varieties were used in most of the experiments.   For the first trial, the nursery trees prior to inoculation were kept under observation for indications of previous infection.   For subsequent experiments, selected variety clons were indexed to determine their freedom from virus infection, propagated on appropriate root stocks and inoculated as nursery stock, usually one year old.   In one experiment, the double budding technique was employed with a healthy clon of Montmorency.   That is, one or more buds from a diseased source were inserted in a *P. mahaleb* seedling, and above these a bud from the healthy clon. Inoculation of named varieties was carried out in duplicate.   The peach seedlings were inoculated, in quintuplicate series, late in the first season of growth.   Two seedlings in each series were cut back to the inserted diseased bud early the next spring, while the remaining three were left intact.

The numbers of different sources of each virus used on the various hosts are listed in table 1.   In a number of instances, individual sources were used in successive experiments, but these duplications are not indicated in the table.

Results

In the several series of experiments, which were carried out over a period of four years, the results obtained with any one host were remarkably consistent. Consequently, to avoid unnecessary repetition, symptoms appearing on the different hosts can be described without reference to individual experiments. These are described in some detail below, and compared in table 2 with symptoms of prune dwarf.

TABLE 2.—*Comparison of symptoms of cherry yellows, necrotic ring spot, and prune dwarf observed on different hosts after inoculation by budding*

Symptoms	Peach varieties and seedlings[a]						Italian prune; Lombard plum			Montmorency cherry		
	A			B								
	NRS[b]	CY	PD	NRS	CY	PD	NRS	CY	PD	NRS	CY	PD
Shock symptoms												
Delayed foliation	0[c]	0	0	1–3	1–3	0–1	0	0	0	1	1	1
Die-back	0	0	0	1–3	1–3	0	0	0	0	0	0	0
Bark necrosis, splitting or roughening	0?	0?	0?	1–3	1–3	0–1	0	0	0	0	0	0
Chlorotic rings and mottle	0–1	0–1	0–1	1–2	1–2	0–1	0	0	0	0–1	0–1	0–1
Etched rings or markings	0	0	0	0	0	0	0	0	0	1–2	1–2	1
Necrotic spotting or flecking	0	0	0	S, F	S, F	0	F	F	0	S, F	S, F	S, F
Chronic symptoms												
Wavy margins	0	1	1	0	1	1	0	0	0	0	0	0
Strap-shaped, pebbled leaves	0	0	0	0	0	0	0	1	1	0	0	0
Shortened internodes	0	1–3	1–3	0	1–3	1–3	0	0	0	0	0	0
Yellowing and leaf casting	0	0	0	0	0	0	0	0	0	0	1–3	0
Recovery	R	r	r	R	Rr	r	R	R	0	R	R	R

[a] Varieties and seedlings in group A were cut back to the inserted bud; those in group B were not cut back.

[b] NRS denotes necrotic ring spot virus; CY, the cherry yellows virus; and PD the prune dwarf virus.

[c] 0 denotes no symptoms; 1, slight symptoms; 2, moderate symptoms; and 3, severe symptoms. F denotes flecking; S, spotting; R, recovery from shock symptoms; and r, partial recovery from chronic symptoms.

Symptoms on peach varieties and seedlings

Necrotic ring spot. On peaches inoculated with necrotic ring spot virus but not cut back to the bud, the first symptom to appear in the following spring was a pronounced delay in foliation, followed by varying degrees of die-back. In many cases, partial emergence of leaves occurred before the branch succumbed; in others, die-back took place before bud movement. Occasionally, two-year-old trees, as well as seedlings, were killed. The earlier formed leaves on the surviving branches frequently,

but not always, showed chlorotic spots or rings, often followed by necrosis. These markings tended to be confined to the basal portion of the lamina. A consistent symptom on one- or two-year-old trees and on the larger seedlings was bark necrosis resulting in a "rough bark" condition, or even in bark splitting, on trunks and scaffold branches. Surviving trees soon outgrew the non-permanent shock symptoms and appeared to develop normally later in the season except that growth was sometimes slightly less vigorous than in healthy trees of the same age. Shortening of internodes or rosetting of shoots was not observed. The shock symptoms did not recur in the second and subsequent seasons, though repeated tests demonstrated that infected trees continued to carry the virus after apparent recovery.

When seedling peach trees were cut back to the inserted diseased bud, they displayed no shock symptoms and proceeded to develop in a more or less normal manner. In other words, in such trees the disease passed directly into the chronic stage without going through the acute stage.

Cherry yellows. If they were not cut back, peach trees or seedlings inoculated with the virus by buds from Montmorency trees affected with yellows exhibited shock symptoms similar to, if not identical with, those induced by necrotic ring spot virus. This reaction occurred whether or not the virus of necrotic ring spot was known to be present in the source trees. On the other hand, instead of recovering, all the peach trees so far inoculated with yellows have produced shoots with shortened internodes. The virus from different sources, however, caused varying degrees of dwarfing, so that, with some, the internodes were only moderately shortened but, with others, were so reduced that the leaves were bunched together in loose rosettes. Variation in dwarfing arising from differences in the varietal reaction of the host have been of minor importance.

When peach seedlings were cut back after inoculation with yellows they also escaped the initial shock symptoms but showed the chronic dwarfing phase of the disease (Fig. 2, A).

Symptoms on plums

Necrotic ring spot. On both Italian prune and Lombard plum, the necrotic ring spot virus from six different sources induced varying amounts of small necrotic spots or flecks on the unfolding leaves, in the spring after inoculation. In one case, however, this symptom appeared in September after budding in August. The necrosis was usually mild, but sometimes was severe enough to give a scorched appearance to the affected leaves. The trees soon outgrew this condition, so that the leaves formed later in the season were normal. There was no recurrence of symptoms in succeeding seasons.

Cherry yellows. The cherry yellows virus from six different sources when transferred either to Italian prune or to Lombard plum caused a similar necrotic spotting of the unfolding leaves at the beginning of the

first growing season after budding. As with trees inoculated with the necrotic ring spot virus, these also soon outgrew the shock symptoms, but, in the second season, branches above the bud insertion produced spindly, stunted shoots bearing roughened or pebbled strap-shaped leaves like those associated with prune dwarf, caused by *Prunus virus* 6. The "prune dwarf" symptoms have increased somewhat in quantity in succeeding years, though spread through the tree has been slow. At time of writing, trees inoculated four years before have only a few branches showing symptoms of this type. Similar results have been observed in Italian prune and Lombard plum inoculated with the milder strains of the prune dwarf virus.

Symptoms on sour cherry

Necrotic ring spot. To date, in these experiments, the necrotic ring spot virus is known, from subsequent expression of symptoms, to have been transmitted to Montmorency cherry from only three sources. In each case, the etched and chlorotic rings and spots, and the necrosis typical of this disease but milder than that observed in nature, were obtained the year following budding. Later the trees appeared to become normal except for a tendency to general thinness of foliage. No symptoms of cherry yellows have been observed on these trees.

Cherry yellows. Cherry yellows virus from eight different sources induced in Montmorency cherries symptoms similar to those associated with the necrotic ring spot virosis. Either later in the same season or not until the second season after budding, typical symptoms of yellows developed. The amount of yellow leaf and defoliation varied considerably from tree to tree.

DISCUSSION

The differential reaction of peaches and Italian prune and Lombard plum to infection with the necrotic ring spot and cherry yellows viruses indicates that these hosts are of considerable value in the diagnosis of these diseases.

It is interesting to note that the symptoms resulting from inoculation with the necrotic ring spot virus are of the same general type, namely necrosis, whether peach, Italian prune, Lombard plum, or Montmorency cherry be used as hosts. Moreover, the sequence of symptoms is similar, namely shock or acute symptoms followed by recovery. That is, chronic symptoms are lacking except for the mild, inconspicuous ones which sometimes recur in Montmorency.

On the other hand, cherry yellows on the same hosts has not only an acute phase, as expressed by shock symptoms resembling those of necrotic ring spot, but also a chronic phase, characterized on Montmorency by yellow leaf and leaf casting; on Italian prune and Lombard plum by

symptoms of the prune dwarf type; and on peach by shortened internodes or rosetting.

In considering the problem raised by the fact that, in its acute phase, cherry yellows on three hosts is indistinguishable from necrotic ring spot, it should be borne in mind that the latter disease is caused by a virus entity distinct from that causing the chronic symptoms of yellows. This conclusion is based on observation of symptom expression on naturally infected Montmorency trees in the orchard as well as on the differential reaction of peach, Lombard plum, and Italian prune to the two viruses. The constant association of the "necrotic ring spot" phase with yellows infection suggests, however, that cherry yellows, if not caused by a single virus, may be caused by a virus complex of which the necrotic ring spot virus is one component.

There remains to be discussed the possible relationship between the cherry yellows and prune dwarf viruses, since the cherry yellows virus from six sources produced symptoms on Italian prune, Lombard plum, and peaches that are indistinguishable from those associated with prune dwarf caused by *Prunus virus* 6 on these hosts (3). This similarity of symptoms would suggest some close affinity between the causal viruses (Table 2). That they are not identical is indicated by the inability of the prune dwarf virus to produce either yellows on Montmorency or typical shock symptoms on peach and plum (2, 8). Accordingly, there would appear to be at least three possible explanations for the similarity and differences in the symptom expression of cherry yellows and prune dwarf:

(1) that the two diseases, though having some symptoms in common, are caused by two distinct virus entities;

(2) that three viruses are involved: one, probably the necrotic ring spot virus, responsible for the acute symptoms; one, the yellows virus, responsible for yellows symptoms on Montmorency and the prune-dwarf-like reaction on peach and plum; and one, the prune dwarf virus responsible for prune dwarf itself;

(3) that cherry yellows is caused by a complex, the components of which are the prune dwarf and necrotic ring spot viruses.

At the present time, the third explanation appears to be the most tenable as it is not probable, though admittedly possible, that two unrelated viruses cause, on the same hosts, symptoms so striking and unusual as those of prune dwarf on Italian prune and Lombard plum. Also, it is not necessary to postulate the existence of a second virus with the apparent characteristics of the necrotic ring spot virus. The evidence now available is not yet sufficient to decide the point, but experiments designed to supply the deficiency are now in progress.

The effect on symptom expression of cutting peaches back to the inserted buds in the spring following budding, as indicated in another connection by Willison and Berkeley (9), is regarded as being due to the

fact that the virosis does not become systemic on peach until after growth starts in the spring. According to this theory, acute symptoms are expressed if infection of the embryonic tissue occurs after differentiation, but only chronic symptoms appear when the embryonic tissues become infected before differentiation. Thus cutting back would remove the upper, non-infected buds and force the growth of adventitious shoots from previously infected tissue in the proximity of the inserted bud. Consequently the shock symptoms of either yellows or necrotic ring spot would appear only on peach seedlings not cut back after inoculation, and seedlings that were cut back would appear to develop normally if inoculated with the virus of necrotic ring spot, or express only the chronic symptoms if inoculated with yellows virus.

SUMMARY

1. The symptoms of necrotic ring spot and of cherry yellows, two viroses common on sour cherry in Ontario, have been described.

2. Inoculation experiments have demonstrated that necrotic ring spot has a short acute phase of the same general type on sour cherry, peach, Italian prune, and Lombard plum and a chronic phase characterized by the absence of symptoms on peach and plum, and frequently on sour cherry, on which, however, mild symptoms occur occasionally.

3. The acute phase of cherry yellows is strikingly similar to that of necrotic ring spot. The chronic phase on sour cherry is characterized by yellowing and casting of leaves in early summer; and on peach and plum, by symptoms simulating those of prune dwarf.

4. The possible relationships between the causal viruses of necrotic ring spot, cherry yellows, and prune dwarf are pointed out.

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A ROOT ROT DISEASE–COMPLEX OF SMALL GRAINS IN VIRGINIA[1]

W I L B E R T A . J E N K I N S

A disease of small grains, herein shown to be one of the root rot disease-complexes, has been responsible for rather severe losses in Virginia over the past 6 years. Highest losses have been observed during late fall and early spring in plantings of wheat, barley, and oats. These losses have formerly been rather consistently attributed to winter injury, drouth injury, or to some more or less obscure phase of foot rot and Helminthosporium blight. In several instances, hasty diagnoses have attributed the trouble to insect injury within the crown of affected plants.

Investigations on root rot disease-complexes of tobacco under way at this Laboratory since 1942 have definitely proved the meadow nematode group to be the basic factor in certain root troubles of tobacco and have contributed materially to a better understanding of similar troubles of small grains.[2]

SUSCEPTS

Though wheat, barley, and oats are the more important of the graminaceous crops cultivated in this area and have therefore received the most attention in this study, identical disease symptoms have also been observed on numerous wild and cultivated grasses in this area. Information is also at hand indicating that numerous crop plants other than Gramineae may be suscepts. Most prominent among these are tobacco and several legumes.

SYMPTOMATOLOGY

When viewed in the late fall and early spring, small grains affected with the root rot disease-complex under consideration appear unthrifty and pale in color when seen from a distance. More or less bare patches are evident throughout the field (Fig. 1, A, B). On closer approach, individual plants over fairly large areas are seen to be stunted or in varying stages of death. Plants typically affected fail to tiller. The older leaves on affected plants have varying color patterns ranging from pale green through bronzing to lemon yellow. Often many dead leaves are to be found about the crowns of such plants (Fig. 2, A, B). Moderately and severely affected plants are generally "heaved" from the soil during periods of freezing and thawing, but it is not unusual for such plants to be uprooted in advance of freezing weather in the fall or following such weather in early spring. Stand losses ranging from 70 to 90 per cent

have been sustained on certain legume-small grain-tobacco rotation plats at this Laboratory, though field losses in general are considerably less. We have no reliable information on the loss in yield.
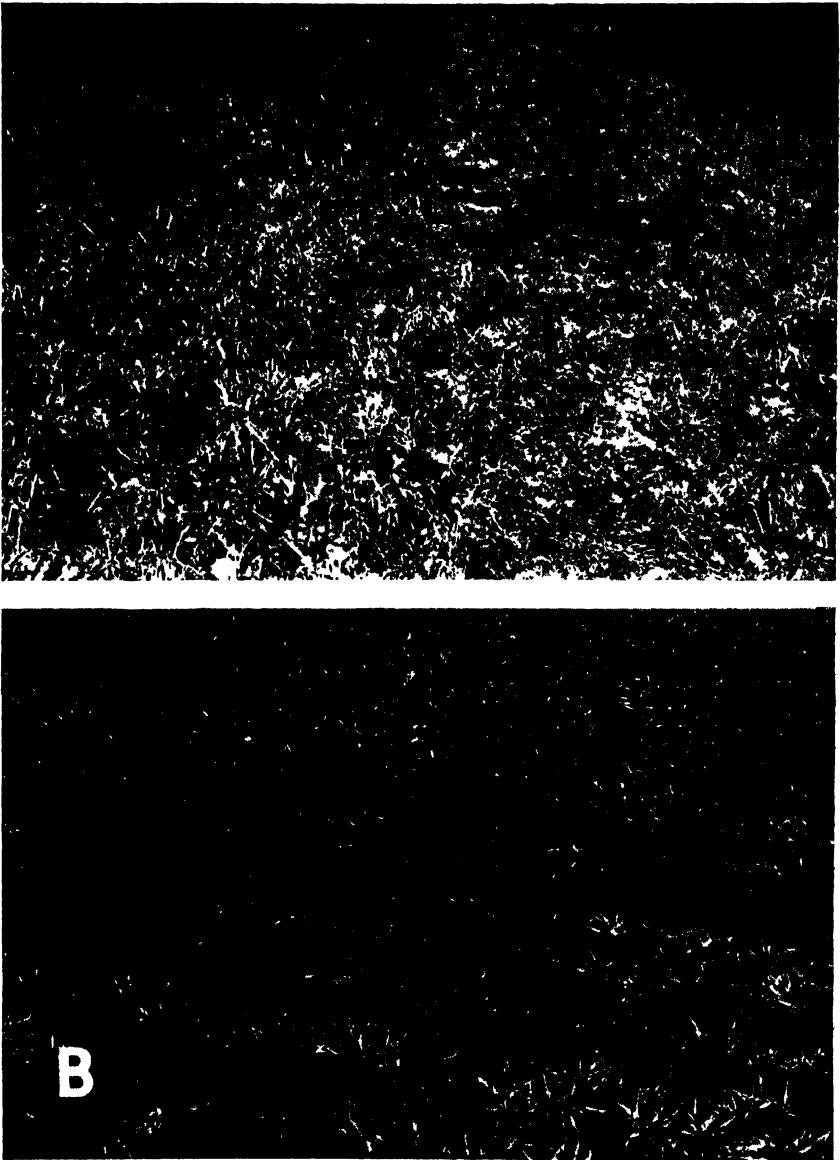
FIG. 1. Field views of small grain fields affected with root rot disease-complex. A. Patterns of destruction within a wheat field; B. Stunting and killing within a barley field.

When individual diseased plants are examined closely, a portion to all of the following symptoms are prominent on the above-ground parts. The

older leaves vary from pale green to lemon yellow, and some are completely dead.   Some of the leaves are almost severed from the plants, the
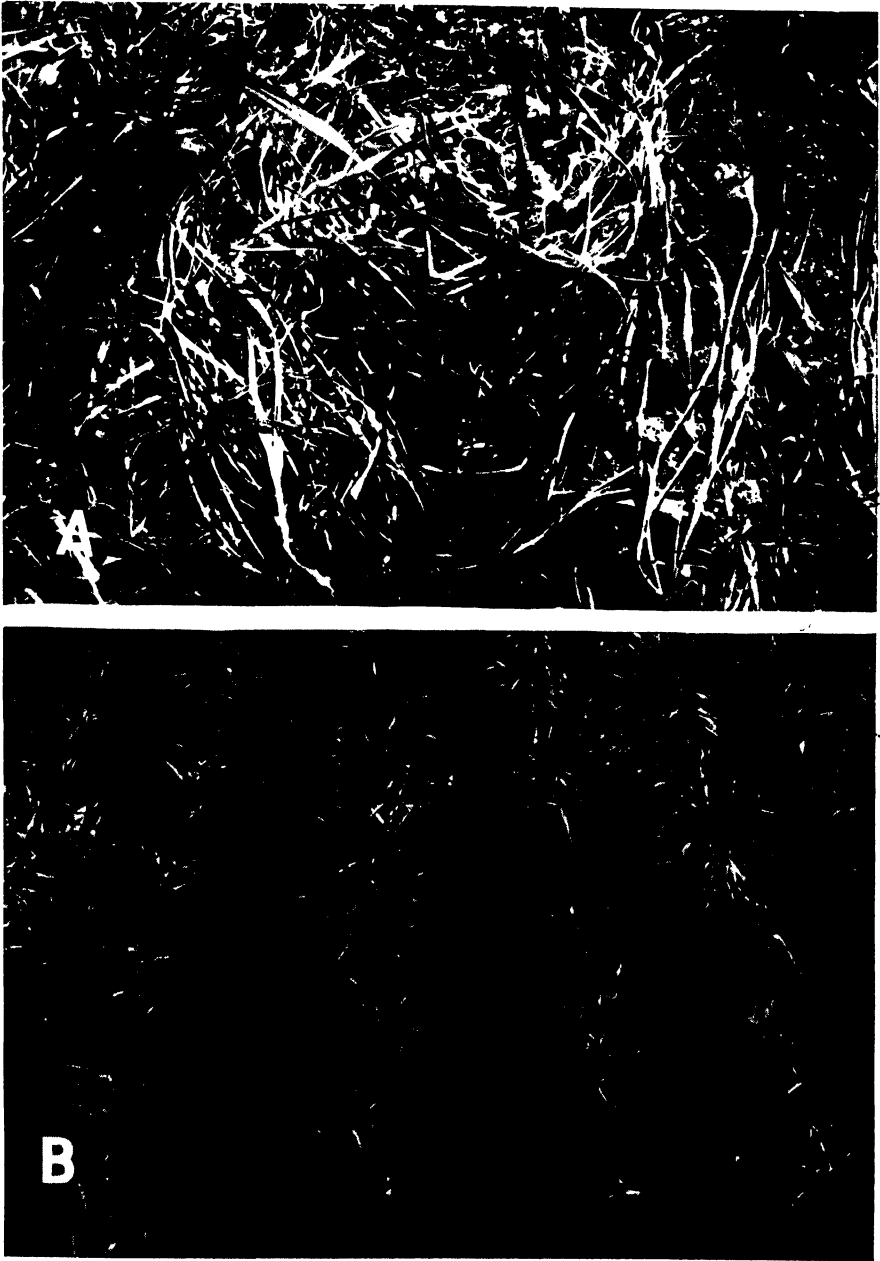
FIG. 2. Close-up view of diseased plants.   A. Wheat;   B. Barley.

impression being that they have been chewed off at or near the basal attachment. .Such leaves readily fall from the.plants when the supporting

soil is removed from about the base. The bases of such leaves present a frayed appearance due to the persistence of the larger vascular elements and quite often, particularly when the soil is moist, pronounced water-soaking occurs in and adjacent to the frayed portions. Severely injured plants often fail to produce seed heads. The inflorescence often fails to emerge from the boot and when it does, the grain is likely to be poorly developed.

Considerable root mutilation is evident in thoroughly washed root systems of affected plants examined under low magnification (Fig. 3, A). All stages between apparently healthy roots and badly mutilated roots can be found depending on the specimen at hand. The earliest indication of root disease is the presence of watersoaked lesions within the cortical tissues. These lesions rapidly change color, becoming pale yellow to tawny

Fig. 3. Photomicrographic views of diseased portions of wheat. A. Extent of mutilation (girdling) of feeder roots; B. Parasitic nemas on and within the leaf tissues of crown; C. Parasitic nema adjacent to a girdle on a feeder root. Note the proximity of many girdles to young and old lateral roots. Roots fixed in Flemming's strong fixative. All photomicrographs × 72 (approximately).

colored as the disease progresses. In time the lesions assume a darker color, attended by a loosening of the cortex and slipping of segments of the cortical tissues in a collar-like or sleeve-like fashion. The underlying stele is various shades of yellow to almost brown in the areas where the cortex is loosened. It is not unusual to find many roots from which all cortical tissues have sloughed off leaving only the woody cylinder.

An examination of thoroughly washed plants, particularly in the crown area, reveals considerable mutilation of leaf bases and young buds. Nematodes may be seen embedded within the leaf tissues when the leaves are stripped from the plants and subjected to microscopic examination. A more accurate picture of infestation may be had by clearing the leaf tissue in warm lacto-phenol and staining lightly with acid fuchsin.

When the crown tissues are separated under suitable conditions and examined under a binocular (dissecting) microscope, many nematodes may be found outside the leaf tissues, but within the folds of the inter-

folded leaves (Fig. 3, B). Preparations for this examination may be made by (1) placing the crown in a preparation dish with a suitable amount of water and teasing the tissues apart under a dissecting microscope or (2) slicing through the crown with a sharp scalpel and then teasing the tissues apart. These preparations may be cleared in lacto-phenol and stained with warm acid fuchsin. Such preparations leave no doubt as to the feeding habits of the nematodes.

<div align="center">ETIOLOGY</div>

Several different nematodes generally are associated with the symptoms described above, the dominant types varying in a large measure with the locality from which the specimens are taken and also with the portion of the plant under examination.

In the vicinity of Chatham and from several locations in the Winchester area of Virginia, the crowns and leaves of small grains are heavily infested with *Panagrolaimus subelongatus* (Cobb) Thorne and *Paraphelenchus pseudoparietinus* (Microletsky) Microletsky. According to Steiner, these two nematodes are usually associated with the type of disease under discussion and *P. pseudoparietinus* particularly appears to be a form with decided preferences for graminaceous hosts. In private correspondence under date of March 31, 1945, Steiner states, "We formerly thought this species an extremely rare one, but find it now everywhere we have opportunity to examine stems, leaves, and seed heads of cereals and grasses."

The roots of diseased small grains in the above mentioned areas are heavily infested with a *Ditylenchus sp.* (exact determination not made by Steiner) closely related to the stem and bulb nematode, and *Pratylenchus spp.*, among the latter being *P. pratensis* (de Man) Filipjev. In some fields *Ditylenchus* predominates, while in others *Pratylenchus* is dominant. These are the nemas which mutilate the feeder roots, thereby initiating a series of more or less complex physiologic and morphologic host reactions which culminate in the state herein designated root rot disease-complex (Fig. 3, C).

During the very early stages of infestation, when the roots show only inconspicuous, watersoaked lesions, no microorganisms can be isolated by bacteriologic techniques—or if cultures are obtained the inconsistency factor is too high to admit the presence of bacterial or fungus parasites. On the other hand, either of the following techniques always demonstrates the presence of nematodes within the lesions. To demonstrate living nemas in situ, the lesions may be teased apart within a limited quantity of water in a Petri or preparation dish under a dissecting microscope. Such roots should first be thoroughly washed in moderately cool water in order to cleanse the roots of any nemas which may be feeding or otherwise attached to the exterior surface of the roots. In order that the thoroughness of the cleansing may be checked, the roots which are to be dissected should be examined closely under a dissecting microscope, following the washing

process.   If nemas are found in the water outside the roots, the cleansing process should be repeated until no traces of nemas are to be found outside the roots.   With such a technique the degree of nema infestation cannot be determined but the method insures the observer against working with free living nemas which may also be in the environment.   If the degree of infestation of roots is to be considered, and no reason is at hand for keeping the nemas alive, another much more rapid method of determination may be used.   This method consists of the early stages of the technique just described, but instead of teasing the lesions apart, the roots containing them are placed in a small quantity of water (just enough water to cover the mass) in a 50-ml. beaker and heated to a temperature just below the boiling point of water.   For practical purposes, this point may be assumed to have been reached when the water begins to send up air bubbles freely, but before the water actually boils.   While cooling, the beaker should be placed on an uneven surface so that the nemas which have emerged from the lesions during the gradual rise in temperature of the water will be concentrated in a given area on the bottom of the beaker.   From this area they can very easily be removed for examination by a small bore pipette and placed in a preparation dish for examination.   Individual nemas may be removed with the aid of a pipette, bamboo splint, or a sharpened broomstraw, and placed on a slide for a permanent preparation.   A very weak formalin solution makes an excellent mounting medium.   Glass wool fibers should be placed within the medium in order to prevent crushing the specimen by the cover glass and each preparation should be sealed with wax in order to prevent it from drying out.

Other associates of root rot complex of small grains, including fungus and bacterial associations, are not specific.   With care, any fungus or bacterial form known to be in the rhizosphere may be isolated from badly mutilated or decaying roots of diseased plants, depending on the moisture content and soil temperature relations during and just preceding the period of examination.   As stated earlier, these bacterial and fungus associates are secondary and under our conditions they will not produce symptoms typical of the disease in the absence of the nemas.

FIELD OBSERVATIONS

Specimens of diseased small grains have been sent to this Laboratory frequently during the past 5 years.   This has afforded an opportunity to evaluate the root rot disease-complex situation over a fairly wide area of the State.   Since much of the small grain grown locally is planted as a fallow crop and therefore not harvested, our principal interest has been to determine if any correlation exists between the incidence and severity of disease in the fallow crop and that of the following tobacco crop.   Considerable time has also been spent in an effort to determine from field observations if any correlation exists between soil type, seed treatments, and fertilizer practices (particularly the rate and frequency of application of lime and nitrogen) and the incidence and severity of disease.

The tobacco growers usually are not particularly interested in the welfare of the fallow crop and since the grain growers have an established program of treatments and rotations, it has been extremely difficult to obtain a productive type of cooperation in the field. The program of soil treatment on a cooperative basis is too recent to yield reliable data.

Under our conditions, root rot disease-complex of small grains is more severe on lighter soils. In a sense, such a situation is to be expected as such soils are usually included in a small grain-tobacco rotation, thus affording a year-around source of food for the nematodes. On those farms planted predominantly to small grains, the general build-up of disease appears to be more gradual.

Field observations also indicate that the severity of the root rot disease-complex may be influenced by seed treatment. The mercury compounds, in general, tend to reduce the incidence and severity of the disease more than do those treatments containing no mercury.

Our most recent field observations on fertilizer practices were made on a farm containing 150 acres of wheat and 75 acres of barley. In those fields which had not been limed within a 2-year period, root rot disease-complex was severe. Fields that had not been limed within the same period, but had received heavy applications of phosphate, were moderately attacked; while fields of both barley and wheat that had been heavily limed the preceding year were only slightly affected. These observations were made during fairly late fall. Observations made on the same fields during early spring, following a 2-week snow cover, indicated no apparent differences between the treatments listed above. All fields were severely affected and losses in stand were heavy.

Other observations indicate that high nitrogen applications noticeably reduce the severity of root rot disease-complex during the first season of its application.

Such observations may not be conclusive, though they have proved consistent over a period of several seasons. Certain practices, including those listed above, give only temporary relief as evidenced by the fact that later crops on the same fields and with the same treatments are often severely damaged by root rot disease-complex. A possible explanation for this anomaly, partially borne out by laboratory observations, is that well nourished plants may produce roots and root generation more rapidly during a given season than the existing nema population can destroy. During the period of apparent control, however, the nema population apparently is built up rapidly in response to an increased food supply and the succeeding crop suffers accordingly.

LABORATORY OBSERVATIONS

The basis of laboratory observations was work done in the greenhouse with infested soils from grain and tobacco fields. Temperature relations received most attention but a few experiments were run on pH levels

(the latter induced principally by high calcium), moisture, and soil amendments. Pertinent data from the tobacco root rot disease-complex studies, which were being run concurrently, were drawn on freely.

Under our conditions, maximum root mutilation developed, other conditions being equal, under conditions of relatively low soil temperatures. Soil temperatures ranging from 68° F. to 73° F. were conducive to maximum feeding by the nemas as evidenced by the severity and patterns of root mutilation. Root degeneration was not excessive at these temperatures. Temperatures below this range were not investigated but judging from field experiences the nemas are voracious feeders at temperatures considerably below 68° F. When soil temperatures were raised to 75° F., and higher, many roots in heavily infested soil escaped severe mutilation. Those roots which were already mutilated at lower temperatures, however, rotted rapidly at higher temperatures, thus indicating the influence of secondary invaders. As the soil temperatures were raised the nemas infesting the roots tended to slacken their feeding cycle and to intensify their reproductive cycle.

In the greenhouse where soil temperature was controlled, root mutilation was significantly more severe in high calcium soils than in low calcium soils. Relative pH readings of such soils did not satisfactorily explain such differences.

Relative moisture content of soils, under conditions of soil temperature control, also exerted a significant influence on the severity of root mutilation. The actual moisture level of the soil, however, did not appear to be more significant than the moisture holding capacity of the soil. In this phase of the work an effort was made to distinguish between the progress of root mutilation and the actual expression of disease symptoms.

In soil flats containing highly infested field soils, severely diseased oat plants made remarkable recovery in those flats to which 10 per cent of DDT (Dichloro-diphenyl-trichloroethane) by weight had been added, whereas those plants reset into untreated soil continued to decline and ultimately died without setting grain.

DISCUSSION

Root rots of small grains have promoted many productive investigations in recent times which have indirectly contributed much toward a better understanding of certain complexities involved. Perhaps no disease is as simple as the relationship between a single parasite and its host and certainly any serious consideration of root rots of crop plants must take into account the dynamic complexities and interactions operating between the root system and the rhizosphere. Since the meadow nemas, as a group, maintain representatives in practically all soil types and tend to play a significant part in the initiation of root troubles in a wide variety of crop plants, it seems they should be accorded much more attention in the future than they have had in the past.

SUMMARY

Small grains affected by root rot disease-complex are unthrifty, stunted, pale in color, and typically they fail to tiller. Older leaves of affected plants exhibit varying color patterns from pale green through bronzing to lemon yellow. When young plants are attacked the leaves become pale and flaccid, and many ultimately shrivel and die. Moderately and severely diseased plants are commonly "heaved" from the soil during periods of freezing and thawing, but it is not uncommon for such plants to be uprooted in early fall and late spring. Stand losses often are severe, affected fields showing bare patches of varying sizes. Moderately and severely affected plants are unproductive.

The root systems of diseased plants, when washed and viewed under magnification, show more definitive symptoms. The earliest indication of root disease is the presence of watersoaked lesions within the cortex of the feeder roots. These lesions undergo rapid color changes, attended by a loosening and slipping of segments of the cortex in a sleeve-like fashion which exposes the woody cylinder.

Leaf bases and young leaves within the crown of affected plants are invaded also and some are actually severed from their attachments.

Root rot disease-complex of small grains has been found to be initiated by the feeding habits of members of the meadow nema group, *Pratylenchus pratensis, Panagrolaimus subelongatus, Paraphelenchus pseudoparietinus,* and a *Ditylenchus sp.* In time and with rising soil temperatures other biologic agents enter injured tissues, both bacteria and fungi, and extensive root rotting occurs.

Control of root rot disease-complex of small grains has not been accomplished but soil fumigation in conjunction with development of small grains that will produce vigorous root systems would appear to have merit.

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ROOT ROT DISEASE–COMPLEXES OF TOBACCO IN VIRGINIA. I. BROWN ROOT ROT[1]

WILBERT A. JENKINS

Brown root rot of tobacco has been known since 1916 when it was reported from the Connecticut Valley, Wisconsin, Maryland, and Kentucky (5, 6). Undoubtedly the disease occurred earlier than this but had previously been confused with other diseases of a similar nature. Johnson (5, 6) has published two classic papers on the subject and several other investigators have added valuable contributions.

Due to the widespread occurrence of brown root rot, as well as to the published work available, most symptoms of the disease are now quite well known to those interested in tobacco as are, also, several conditions which influence the incidence and intensity of the disease.

Earlier work (3, 5) sought to demonstrate a fungus or bacterial pathogen but these efforts proved fruitless. Lehman (7) published an excellent description of a "new" brown root rot disease of tobacco from North Carolina with which *Tylenchus pratensis* de Man (*Pratylenchus pratensis* (de Man) Filipjev) was associated. Although the causation of the disease was not proved experimentally, there seems little doubt but that the meadow nema was regarded as a primary parasite by this investigator. He also determined that relatively high calcium levels in the soil, as measured in terms of rates of application of lime and pH determinations, favored the intensity of the disease. Lehman concluded that this disease and that described earlier from Wisconsin were not of the same origin, his premise being that the intensity of brown root rot diminished with successive crops of tobacco in Wisconsin, whereas the intensity of the "new" brown root rot increased in intensity with successive tobacco crops in North Carolina. Steiner (9) supplemented the above report by publishing a taxonomic determination of the nemas associated with root rots of tobacco, noting that tobacco was a new host for *T. pratensis*.

A vast preponderance of the published work relates the causation of brown root rot to certain non-living soil constituents arising directly or indirectly from decomposition of residues from preceding crops (2, 5, 6, 10). This interpretation of the etiology, though admittedly tentative, evoked surprisingly little controversy considering the rather abstruse nature of the hypothesis until other investigators again sought to prove the relationship of a fungus pathogen (8).

Work under way at this Laboratory since 1941 definitely established brown root rot as one of the root rot disease-complexes initiated by the feeding habits of members of the meadow nematode group and resulted in the publication of a preliminary report (4). Partial confirmation of our

results has since been published (1, 11). A more complete exposition of
the results accomplished at this Laboratory forms the basis of the present
report.

<center>SUSCEPTS</center>

Brown root rot is known to affect the several types of tobacco generally
grown in Wisconsin, the Connecticut Valley, Kentucky, and Maryland
(5, 8). Such types as dark fire-cured and burley are known to be affected
in Virginia, Tennessee, and Kentucky while flue-cured types are affected
in Virginia, North Carolina, South Carolina, and Georgia. There are
persistent reports of brown root rot in flue-cured tobacco in the Province
of Ontario, Canada, and in shade grown tobacco in Florida, as well. Other

FIG. 1. Field view of Yellow Special strain of tobacco affected by root rot disease-
complex. The field was adequately cultivated but the plants never attained sufficient
growth to shade out the grass and weeds. Note the ''cabbage-like'' growth, with wilting,
stunted, bony leaves. Two successive crops were complete failures on this field.

solanaceous crop plants such as tomatoes, potatoes, eggplant, and pep-
per, as well as several leguminous and graminaceous crop plants were
reported to be more or less severely affected (6). Our investigations con-
firm this diverse host range in so far as members of the above list have
been investigated. In addition, we have found crab grass (*Digitaria* spp.)
and *Buxus* spp. (boxwood) to be seriously affected and *Vitis* spp. (common
bunch grapes) and corn[4] (*Zea mays*) moderately to severely affected.
Since members of the meadow nema group are universal in distribution and
are voracious feeders, it is highly probable that a study of a vast num-
ber of root rot disease-complexes, which at present are attributed to other
causes, may in time contribute to an extension of the list of suscepts for
brown root rot.

SYMPTOMATOLOGY

One of the outstanding above-ground symptoms of brown root rot in the field is its resemblance to the disease generally known as black root rot. It is unusual to find all plants in a given field severely affected, though this sometimes happens (Fig. 1, Fig. 3, A). As a rule, symptoms are severe in areas of varying size within a given field, whereas sections of the same field may appear only moderately if at all affected. Quite often severely affected plants appear individually and at random throughout a field.
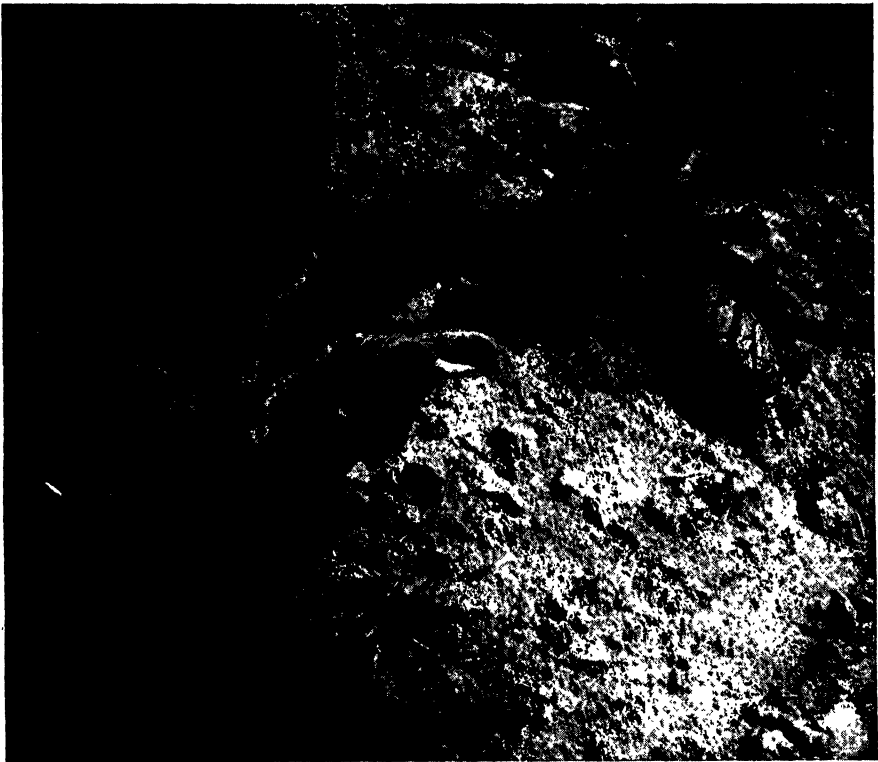
FIG. 2. Typical symptoms of root rot disease-complex on young tobacco in the presence of adequate soil moisture. Note the severe wilting of the leaves and the beginning of "cabbage-like" growth.

Plants severely attacked have symptoms of severe drouth even in the presence of adequate moisture (Fig. 2). Such plants develop a "cabbage-like" type of growth, entirely uncharacteristic of flue-cured types. When seedlings are attacked they generally develop wiry stems and rarely recover. During periods of drouth or excessive water loss, even moderately affected plants develop symptoms of a severe type. Plants thus affected become stunted throughout and the leaves tend to undergo successive periods of wilting. It is not unusual for the entire distal portions of such

leaves to burn off completely, thus ruining a third to half the leaf area. Leaves of such plants, become dwarfed and the larger vascular elements tend to stand out in coarse rib-like fashion (Fig. 1). Pronounced flagging of such leaves is common, followed by premature yellowing and rim-firing of the older leaves (Fig. 3, A and B). Needless to say, such leaves must be harvested green, if at all, thus resulting in a poor quality product and heavy loss to the grower.

Brown root rot symptoms are more definitive on the root systems of affected plants than on the above-ground portions. Here the true nature
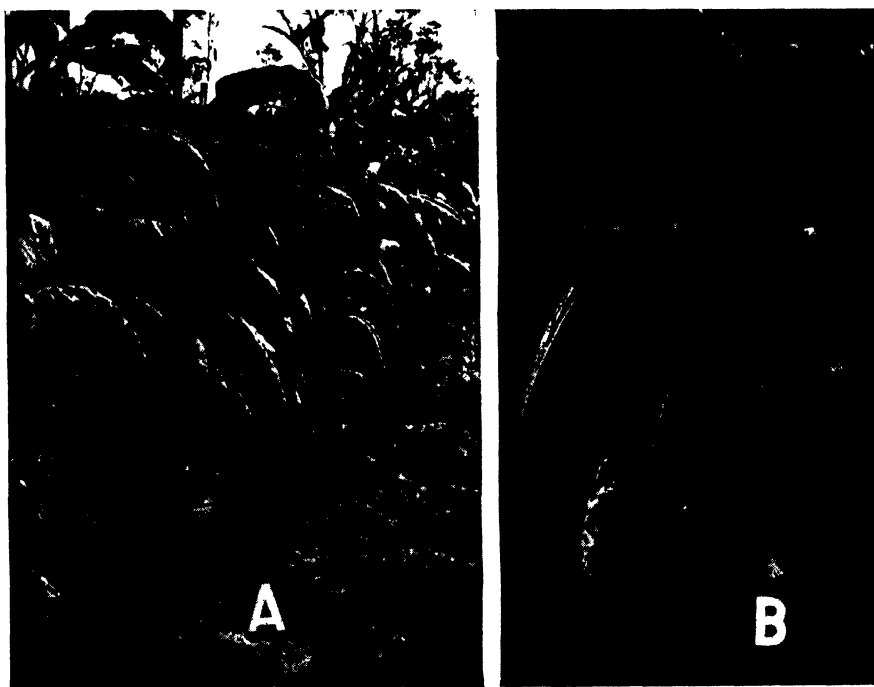
Fig. 3. Typical field symptoms of root rot disease-complex on mature tobacco. A. Portion of a 4-acre field of Bonanza which yielded about 300 sticks of tobacco that would not cure. B. Close-up view of severe rim-firing and tip burning of the older leaves.

of the disease may be ascertained. Severely affected plants may be easily pulled from the soil by midsummer because of almost complete destruction of the root systems. Moderately affected plants exhibit varying stages of root survival, but generally the individual roots are discolored, stubby, and surviving roots are grouped near the soil surface (Fig. 4). Under magnification, affected roots show varying degrees of mutilation consisting of cortical lesions varying from pale yellow to almost black. Such lesions generally result in complete girdling of the feeder roots followed by loss of the cortical tissues through sloughing off (Fig. 5, A, B). The incidence and severity of root decay depends on such factors as soil tempera-

ture, moisture, calcium level, and the population of biologic associations in the rhizosphere. The above mentioned factors also influence the color of the deteriorating roots so that brown root rot is not an altogether descriptive designation for the disease.

A considerable amount of field work has been accomplished on brown root rot at this Laboratory but our most reliable data have been secured in the greenhouse and laboratory under controlled conditions.

Fig. 4. Root system of a plant affected with root rot disease-complex. Note the "brush" of roots remaining nea- the soil line and the bare roots below. Even the roots near the soil line show evidence of severe mutilation.

MATERIALS AND METHODS

The basic results of the present investigation were secured from soils taken from fields that had produced essentially worthless crops of tobacco over a period of several years because of brown root rot. Preliminary examinations had shown these soils to be free from *Thielaviopsis basicola* (Berk.) Ferraris, but heavily infested with meadow nematodes, of which *Pratylenchus pratensis* (de Man) Filipjev was the dominant component of the population. Soils were also taken from the black root rot plots at this Laboratory which have consistently produced stunted crops of tobacco over a period of 15 years when planted to susceptible strains. This soil was known to be heavily infested with certain members of the meadow nematode group, except *P. pratensis*, as well as with several different fungi, including *T. basicola*. Other soils were taken from the agronomy plots at this Station on which profitable crops of tobacco have been grown since 1936 and from which there had been no complaint regarding yield and quality. This latter soil had been cropped continuously with small

grain-tobacco rotations, the small grain being used as a winter cover crop. For benefit of later discussion, the soil from the brown root rot fields is designated I and is Appling type; soil from the black root rot plots is designated II and is Cecil Clay Loam type; while soil from the agronomy plots is designated III and is Granville type.

These soils were used in one-gallon, glazed pottery crocks, suitably set up for proper drainage and aeration of the soil. Fertilizer, 3–9–6 analysis, was used at the same per plant rate as in the field. Check lots of all soils were sterilized within the crocks in an autoclave at 20-lb. pressure for 2 hours. Plants grown in pots until well rooted at greenhouse temperatures were transferred to constant temperature tanks equipped with both re-
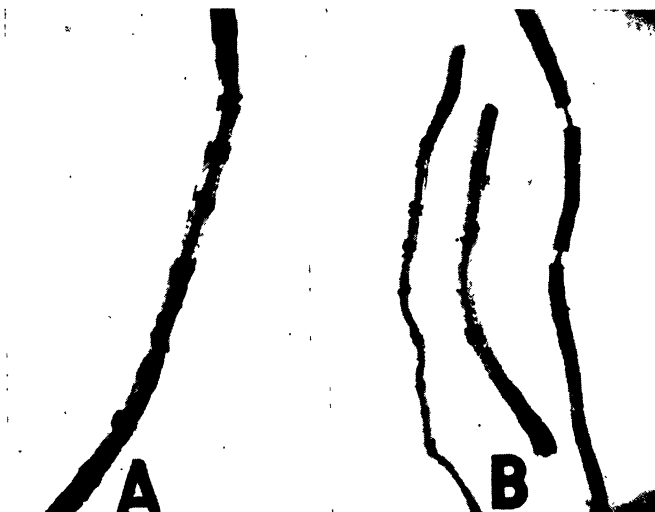
Fig. 5. Photomicrographic views of mutilated feeder roots from plants affected with root rot disease-complex. A. Extensive girdling of feeder roots and discoloration of tissues by biologic associates, in this instance, *Thielaviopsis basicola* among other fungi. B. Transition between girdles and sloughing of the cortical tissues.

frigeration and heating units. By means of dual thermoregulators, the soil temperatures were maintained within 1° F. (±) of a selected point over a temperature range of 32° F. to 120° F. Air temperature was controlled through ventilation of the greenhouse and soil moisture was regulated manually. Since the tanks were not enclosed, the above-ground portions of test plants had benefit of full sunlight, conditioned only by the glass roof of the greenhouse. By means of auxiliary lighting, the photoperiod was maintained equivalent to that of early spring conditions in the field. Seedlings were grown to transplanting size in steam sterilized sand flats. The test strains of tobacco were Yellow Special (highly tolerant to several root rot disease-complex manifestations and generally considered highly tolerant to black root rot), Yellow Mammoth, and White Stem Orinoco (both highly susceptible to root rot disease-complexes, including black root rot).

EXPERIMENTAL RESULTS

Soil temperature studies. In an effort to duplicate the soil temperatures prevailing in this area at the time the crop is normally set into the field, seedlings of each test strain were grown at several different temperatures, and some were started at one temperature and later subjected to a higher temperature. At the end of a 10-day period, plants were removed from soils maintained at approximately 68° F., the root systems washed thoroughly and examined under a dissecting microscope. Root samples were also preserved for further study. In some instances, random lots of seedlings were reset into the crocks and grown for additional intervals in order that the course of development could be studied at later dates. Additional plants were removed from all soils at varying intervals following the 10-day period so that the progress of events could be traced throughout the growing season.

When examined within 10 days following transplanting, numerous uncomplicated lesions were found on all young roots regardless of soil type and variety. The earliest distinguishable lesions were present on practically all portions of the roots except in the growing points and in the relatively mature tissues. As a rule, most lesions and the more severe early symptoms of root mutilation were present in the region of elongation. Lesions within the growing points were not seen.

The lesions are initially devoid of pigment but may appear slightly elevated and watersoaked under magnification. These are the lesions which yield nematodes in the largest numbers. Very soon after formation a majority of the lesions break open because of separation·of the injured cortical tissues and the rupture extends into the cambial region. Probes inserted into young lesions, as well as transverse sections, confirm the observation that the nematodes penetrate through the cortex and into the cambial layer. This indicates that the nematodes may feed within the cambial tissues. Observations also indicate that the lesion is initiated by the insertion of the nematode stylet and that further rupture of the injured area may be due to high cellular tension existing at the point of penetration.

With age, the lesions become progressively more discolored. The earliest color change is to pale cream or pale yellow, finally developing a reddish to black appearance, apparently depending somewhat on the nature of the biologic successions. Lesions are found quite often over the potential exit of a secondary root or in the crotch of a lateral root. Most lesions eventually result in the separation of segments of the cortical tissue from the woody cylinder at the cambium. This condition is followed by sloughing off of the cortical tissues in a sleeve-like manner. Lesions uncomplicated by root rotting associates generally cause affected roots to shrivel and die, particularly if the root is a feeder root and lesions are numerous. Lesions·formed on older root tissues often heal over, though

the callus remains evident as prominent scar tissue. This activity results in extensive root pruning. Continuation of root generation coupled with root pruning is responsible for the stubby appearance of affected root systems. Since the feeding habits of the nematodes are markedly affected by temperature, as will be brought out later, the higher temperatures near the soil line may afford a measure of protection for adventitious roots developed in this area, thus explaining the survival of some roots near the soil line.

At temperatures of 73° F. to 80° F., initially, lesion formation is accompanied by several biologic associates other than nematodes, which complicates the picture. Under such conditions, extensive root rotting may occur rather early. Plants started at 68° F. and then subjected to higher temperatures (75° F. to 80° F.) give us a better understanding of what is happening.

In soil I, the nematode which is dominant at 68° F. is also dominant at temperatures between 73° F. and 80° F. Lesions are formed in a measured pattern at 68° F. (Fig. 4) and this pattern is continued at higher temperatures. At temperatures above 75° F. there is a distinct tendency toward more longitudinal movement by the nematodes in affected roots, however, and the reproductive processes of the nematode apparently are accelerated. Root rotting also is enhanced by the higher temperatures.

In soils II and III the entire picture is altered by elevation of the soil temperature. At 68° F. many lesions are formed but the pattern is variable, i. e., the lesions do not appear at measured intervals along the roots. At the same time, several different nematodes are present in the soils with little evidence that any one species is dominant. The picture is even more confusing with successive crops of tobacco because the specificity of the nematode population fluctuates. Under these conditions, there appears to be a succession of nematode forms resulting in specific alterations in the nematode complement of the soils. When the temperature of these soils is raised into the range of 75° F. to 80° F., essentially all nematode feeding ceases. Eggs are laid but for some reason new penetrations diminish in frequency and the plants tend to recover. The roots that were badly mutilated at 68° F. decay rapidly at high temperatures, but the rotting does not spread to new roots.

In soil II, *Thielaviopsis basicola,* among other fungi, grows luxuriantly on, and adjacent to, the lesions and sloughing cortical tissues, but as the soil temperatures are elevated to the point where nematode feeding diminishes, new loci of infestation by *T. basicola* diminish proportionately.

The above-ground portions of all varieties were stunted severely on soils I, II, and III at 68° F. At temperatures of 80° F. or higher, all tobacco strains on soil I remained severely stunted, but on soils II and III the strains were easily separable on the basis of tolerance. All strains attained more height on soil III than on soil II at 68° F.

At 68° F. on soil II, Yellow Special attained significantly more height than did Yellow Mammoth or White Stem Orinoco, but it did not approximate the height of the check thus indicating that the strain was stunted by root mutilation. At temperatures of 80° F. and higher, Yellow Special attained significantly more height than did either of the other strains under test. At least some portion of this response was due to the ability of Yellow Special to continue root generation, whereas the other two strains showed evidence that they were incapable of sustaining a high rate of root generation under the same conditions.

All strains grew normally on the check (sterilized) soils of all types. There was very little root mutilation in the checks and the small numbers of nematodes found were indicative that a slight infestation was introduced by splashing water or by air currents.

It thus appears that injury at all soil temperatures results from an unbalance between the rate of root mutilation by the nematodes and the rate of root generation by the host. At low soil temperatures (68° F.) the nematodes are voracious feeders. Since root generation is comparatively slow at such a temperature, the feeder roots are destroyed faster than new roots are generated, and the result is stunting of the plants. At higher temperatures, the reproductive phase of the nematode is accelerated and feeding slows down. Whether or not the plants generate roots faster, the plants tend to recover because of a more favorable balance. The exception to this general statement is found in the case of plants affected by *Pratylenchus pratensis*. In such plants there is no recovery or it is only slight.

Studies with air-dried soils. During the early phases of this investigation, soils were collected from many different fields where tobacco had symptoms of brown root rot. Often the soils became completely air-dried before they were used. It was soon noted that such soils consistently failed to induce typical symptoms of brown root rot when seedlings were set into them. Since this experience was in accord with Johnson's findings (5), no effort was made to elaborate on this phase of the work. It was determined, however, that *Pratylenchus pratensis* is more susceptible to destruction from drying than are certain other nemas associated with it in many tobacco soils collected in eastern Virginia and eastern North Carolina.

Studies with chemical amendments. Having determined the probable causation of brown root rot, together with several climatic factors favoring its severity, it was thought that practical control of this and other root rot disease-complexes might be achieved by affording transient protection to susceptible strains of tobacco long enough for seedlings to recover from the shock of transplanting and generate a functioning root system. This idea was based on the hypothesis that a tobacco plant might create a more favorable balance for its feeder root system through root generation if the rhizosphere were rendered temporarily free of nemas at the time of transplanting.

Chemical treatments were used as dust and liquid formulations and the root systems of the plants were plunged into the chemicals immediately before being set into the soil.

Of the many chemicals and formulations of each that were used, only a very few satisfied the conditions for which they were intended. For instance, a vast number of the materials containing mercury were too toxic to the root systems even though they proved highly effective against the nemas. Benzine hexachloride (Hexachlorocyclohexane) was in the same category with mercury. Varying formulations of DDT (Dichloro-diphenyl-trichloroethane) from 10 per cent to 50 per cent by weight gave excellent control in certain fields and in the greenhouse. In other fields, however, DDT treatments were not effective, probably because the effects were too transient.

Limited information was secured in greenhouse cultures indicating that a high calcium level in the soil definitely favors brown root rot. This effect is particularly noticeable in the crop following that to which the application was made. High nitrogen levels gave very similar results. In the greenhouse experiments, the calcium level was roughly equivalent to an application of 150 lb. lime per acre to ordinary tobacco soil and the nitrogen level was roughly equivalent to a side dressing of 50 lb. nitrate of soda per acre on ordinary tobacco soil. Such information means little by way of field application since conditions in the field soils as influenced by past practices would vary more widely.

ETIOLOGY

Isolations from very young lesions on root systems grown on all soil types at 68° F. were either negative or the inconsistencies and variation among the isolations were so prevalent as to preclude the possibility of a primary bacterial or fungus parasite when bacteriologic methods were used. On the other hand, members of the meadow nema group were abundant in the very young lesions when the proper method of demonstrating their presence was used. Representatives of all fungus and bacterial isolates were incapable of inducing symptoms of brown root rot when used as inoculum, except in the presence of members of the meadow nema group.

In material from soil I grown at 68° F., *Pratylenchus pratensis* is the nema species found almost exclusively. At higher temperatures in the same soil, *P. pratensis* continues to be the dominant nema present, though other nemas as well as fungi and bacteria may be readily isolated from decomposing roots.

Materials from soils II and III grown at 68° F. yielded relatively large populations of nemas of several types, including *Ditylenchus sp.*, *Aphelenchoides sp., Panagrolaimus sp., Aphelenchus sp.,* and *Acrobeloides spp.* In these soils no single species predominated at any temperature. Bacterial and fungus isolations were negative from very young lesions. As the lesions began to rupture and adjacent tissues deteriorated, numerous

fungi and bacteria were isolated. As in soil I, isolations from soil II and III varied in almost direct proportion to the time interval the roots had been exposed. During the initial 10-day interval comparatively few species of fungi and bacteria were isolated, but at successive intervals the numbers increased. Such a pattern of increase might readily indicate normal biologic succession. For instance, in soil II, the earliest fungi isolated were species of *Fusarium* and *Pythium,* but as time intervals multiplied, *Mucors, Macrophomina phaseoli, Thielavia basicola,* and *Thielaviopsis basicola* were readily isolated.

When the temperature of soils II and III was raised to 80° F. and higher, the nema population began to fluctuate widely, finally resulting in an entirely different nemic population from that originally present. At these higher temperatures feeding by the nemas (as evidenced by lesion development) ceased. At the same time, *Thielavia* and *Thielaviopsis* disappeared from the roots, though *Thielaviopsis* could still be readily isolated from the soil by appropriate methods (12).

DISCUSSION

The etiological aspects of brown root rot of tobacco are no longer obscure, but a consideration of the available data develops a concept of plant disease which is difficult to discuss except on a broad basis.

An evaluation of the data which have accumulated through work accomplished under controlled conditions demonstrates rather conclusively that brown root rot of tobacco must be classified as a root rot disease-complex. Our experiences demonstrate that the disease in this area is initiated by the feeding habits of members of the meadow nema group and that one of these nemas, *Pratylenchus pratensis,* is alone capable of inducing symptoms typical of the disease.

In our work with various strains of flue-cured tobacco grown under controlled conditions on soils known to produce consistent symptoms of brown root rot in the field, very young lesions yield essentially negative isolations, except for *Pratylenchus pratensis,* when the soil temperature is maintained at 68° F. At higher temperatures, isolations of fungi and bacteria from deteriorating roots are too inconsistent and variable to indicate more than normal biologic succession in decomposing tissues. When representatives of these isolations were reinoculated into clean soil, no symptoms of brown root rot develop on tobacco grown in it; whereas the disease always develops in the presence of *P. pratensis.*

Soils taken from fields in this area invariably harbor large populations of nemas. A vast proportion of this nema population feeds on living roots of tobacco and other crop plants, as well as weeds, growing in the soils. Since the nemas prefer succulent tissues, it is only natural that the feeder roots will suffer most, regardless of the crop in question. Such a situation brings very sharply into focus the problem of a potential reduction in vegetative vigor in the exposed crop with consequent reduction in yields

and quality. This may and undoubtedly does occur over wide areas even in the absence of specific symptoms on the above-ground parts of affected plants. The implications of such a situation are tremendous both from the agronomic and pathologic standpoint, for complications arising from mutilation of the feeder root system of a crop plant may vary all the way from symptoms of nutritional deficiencies to those of an epiphytotic disease.

The matter of biologic associations has merely been mentioned, not explored. Certain nemas may function both as primary invaders and as carriers of biologic associates, some of which may, following introduction, be classified as primary parasites and a majority of which may attack tissues already weakened by mechanical mutilation. Thus transient diseases may be due to nemas which are non-toxic and which respond in their feeding habits to very narrow temperature ranges.

The physiologic or toxicity effects of nema feeding on tobacco roots has not been investigated but observational evidence suggests that certain species, among these being *Pratylenchus pratensis*, are highly toxic to certain crop plants over and beyond the matter of root mutilation. Other forms which show little evidence of toxicity undoubtedly bring about mild to severe nutritional deficiencies in crop plants by mechanical destruction of roots, while others may act as carriers of saprophytic or parasitic associates, perhaps including some of the viruses.

Another very interesting aspect of root rot disease-complex brought out by this work is the fact that tobacco strains of different genetic constitution often respond differently to nema invasion. The best evidence for this opinion rests on comparative measurements of plant height, for our results show that a given strain may suffer significantly less stunting of the above-ground parts than another although the root systems of both may be about equally mutilated. Where soil infestation by *Pratylenchus pratensis* occurs, however, the situation is basically different. We have found general indications that a strain of different genetic constitution will often produce a satisfactory crop in the presence of *P. pratensis* provided this strain is being grown on the infested soil for the first time, i.e., following a long period of culture of some other genetically different strain. These results appear to follow the principle of adaptive feeding by the nemas. In time, one would expect the nemas to become adapted to any new introduction.

Calcium and nitrogen levels appear to exert both a direct and indirect effect on the nema populations. Calcium, for some reason, appears to favor the nemas directly, whereas both calcium and nitrogen encourage the development of extensive root systems which in turn might be expected to influence the increase and accommodation of larger nema populations. That this may be true seems to be borne out by the fact that brown root rot and other root rot disease-complexes of crops appear to be more severe on crops following those which received supplemental applications of calcium and nitrogen.

Finally, host adaptation would appear to offer a valid explanation for variations in intensity of brown root rot of tobacco in different parts of the country as related to cropping practices. Our experience with members of the meadow nema group indicates that members of the grass family are more natural hosts of the group as a whole than is tobacco. Consequently, in the flue-cured tobacco belts where successive tobacco crops are grown for long periods on the same fields, the nemic populations consist of large numbers of highly adapted individuals which persist in accordance with the cropping practices. In other sections of the country where tobacco is grown periodically following more or less lengthy rotations with grasses and other crops, large populations of nemas undoubtedly develop and persist but the adaptive process toward tobacco is not so acute. Since, under the latter conditions, grasses would appear to be the favored host, the nemic population on a given field would be expected to diminish with each successive tobacco crop grown on a given field, at least until such time as that population increase could be initiated and sustained by adapted individuals. By host adaptation is meant the ability of the nema to reproduce successfully on a given host, not merely the facility of feeding on it.

SUMMARY

Studies of tobacco root rots in the field and under controlled conditions in the greenhouse and laboratory since 1942 offer conclusive evidence that brown root rot should be classified as one of the root rot disease-complexes.

Tobacco affected by brown root rot has unmistakable symptoms of drouth injury. In general, affected plants do not grow properly following transplanting, either in certain areas within the field or throughout the field. The leaves of affected plants undergo alternate periods of wilting and recovery during early stages of the disease, but as the season progresses many leaves fail to regain turgidity wholly or in part, resulting in stunted plants bearing coarse, stunted leaves with varying degrees of tip burning and rim firing. Severely diseased plants frequently have symptoms of plant food deficiencies.

Brown root rot symptoms are more definitive on the feeder root system which typically becomes mutilated to such an extent that it can no longer adequately perform its function. Lesions, as seen under magnification, are at first watersoaked to pale yellow and finally brown to black. The color changes generally follow rather extensive cortical fissures (girdles) which extend into the cambial zone and result in extensive sloughing of the cortex. Root rotting often accompanies root mutilation but varies in extent and severity with such factors as soil temperature, moisture, calcium level, and biologic associates in the rhizosphere. Root mutilation precedes root rotting and may, alone or in conjunction with the latter, result in an inadequate feeder root system.

Under our conditions, brown root rot of tobacco is initiated by the

feeding habits of members of the meadow nematode group, of which the true meadow nema, *Pratylenchus pratensis* (de Man) Filipjev consistently produces severe symptoms.

Adequate control of brown root rot has not been attained. Such measures as supplementary nitrogen applications, rotations with tobacco varieties of different genetic constitution, and hilling the soil high about the bases of plants have afforded temporary benefit to the current crop. Soil fumigation studies recently begun appear to offer more permanent control when used in conjunction with varieties of tobacco developed to produce more vigorous root generation.

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THERAPEUTIC TREATMENTS FOR RUSTS

C . E . Y A R W O O D

Several exceptions are known to the common generalization that diseases caused by rust fungi are not subject to control treatments after infection has occurred. Newton *et al.* (3) found that catechol injected into wheat leaves 4 days after inoculation with rust, reduced subsequent rust development by about 50 per cent. Gassner and Straib (2) reported that sulphur dust was effective in reducing cereal rust when applied up to 10 hours after inoculation and kainite up to 48 hours after inoculation. Gassner and Hassebrauk (1) discovered that overnight immersion of leaves in sodium sulphide solutions killed cereal rust infections up to 3 days after inoculation. Sempio (7) found that temperatures of 34° to 36° C. for 2½ days completely killed bean rust mycelium when the treatment was applied 4 days after inoculation. Ogilvie and Brian (5) developed a practical control of mint rust by treating the cuttings for 10 minutes in water at 112° F. but the nature of the control is not clearly set forth, and Niederhauser (4), who confirmed the success of the method, believed that control resulted from the killing of the uredospores and not from the killing of the rust mycelium in the tissues as it is to be inferred from the results of Brian and Ogilvie. Parker-Rhodes (6) used 15 minutes immersion in water at 40° C. for the eradication of two wheat rusts, but no details of rust development are given. Yarwood (9) reported killing of 5-day-old uredial pustules of bean rust, snapdragon rust, and sunflower rust without host injury by exposure of the plants to the vapors from lime sulphur solutions, or from dilute hydrogen sulphide gas. Zaumeyer and Goldsworthy (11) state that liquid lime sulphur, chlorinated naphthoquinone, and disodium ethylene bisdithiocarbamate were effective in eradicating 24-hour infections of bean rust. The present report presents quantitative details of rust therapy by the above and other methods.

MATERIALS AND METHODS

The uredial stages of sunflower rust (*Puccinia helianthi*) on variety Mammoth Russian, bean rust (*Uromyces phaseoli*) on variety Pinto, snapdragon rust (*Puccinia antirrhini*) on varieties 'of cultivated snapdragon, and mint rust (*Puccinia menthae*) on spearmint were used in these tests. The rusts were collected in the San Francisco Bay region and all tests were made on plants growing in 4-inch pots in the greenhouse at Berkeley, California. Pinto bean plants with paired primary unifoliate leaves suitable for inoculation in 8 to 12 days from seeding were best suited for these tests and were most used. The plants were inoculated by spraying them with a spore suspension and incubating them overnight, usually about 14 hours, in moist chambers with a prevailing temperature of about 17° C.,

and then placed on the greenhouse bench. Tests of eradicant (therapeutic) treatments were usually made at 4 days after inoculation, at which time the pustules first became evident, but shorter and longer infection periods were also used.

To test the eradicant action of dry air, as a measure of the incubation (in the sense of Whetzel) period under conditions of these trials, inoculated plants, placed in moist chamber incubators at inoculation, were removed from the moist chambers at varying times after inoculation and placed on a turntable in the greenhouse, where the leaf surfaces usually dried in about 30 min. Soon after the plants had dried, they were returned to the greenhouse bench.

Spraying was done with a No. 15 DeVilbiss atomizer attached to 35 lb. air pressure. For ordinary coverage applications the nozzle was held about 8 inches from the leaves to be sprayed, but for the penetrating or water soaking applications the nozzle was held at about ½ inch from the lower leaf surface. A spreader, 0.05 per cent B1956, a proprietary form of phthalic glycerol alkyl resin, was used in all cases where a spreader is indicated.

Infection was normally measured by counting the living pustules per square centimeter at about 10 days after inoculation. With the beans, untreated check leaves were the twin leaves opposite the treated leaves, except in the tests with dry air, when, as with all tests on all hosts other than bean, the check leaves were on separate plants. Infection on the checks ranged from 20 to 100 pustules per sq. cm. and percentage control was calculated as

$$\frac{\text{Pustules per cm.}^2 \text{ on check} - \text{Pustules per cm.}^2 \text{ on treated} \times 100}{\text{Pustules per cm.}^2 \text{ on check}}$$

In a few cases, as in the hot water treatments, practically all pustules in a given treatment reacted alike, eradication was total or nil, and intermediate values were estimated.

In trials of vapors, as those from lime sulphur solution and from mixtures of sodium cyanide and sulphuric acid, exposures were of two types. In most tests the leaf to be treated was placed in a 450-cc. fruit jar with a notch in the rim to accommodate the petiole. After the leaf lamina, still attached to the mother plant, was placed in the jar, the glass top with rubber seal was fastened on with a rubber band and the opening around the petiole was sealed with modelling clay. Plants with leaves in jars of this type were placed on a rotating turntable in daylight for the specified exposure period and then the leaf was removed from the jar and the plant returned to the greenhouse bench. In another type of exposure the pots of plants to be treated were placed in closed 120-liter cans in the dark and the sodium cyanide-sulphuric acid mixture in a jar was placed beside the plants, but with no provisions for forced air circulation.

Cyanide was produced by adding an equal volume of 20 per cent sul-

phuric acid to the required amount of 0.2 per cent sodium cyanide. It is assumed that all the cyanide was released as gas.

To test the therapeutic effect of hot water on bean rust and mint rust, infected leaves of potted plants were immersed in distilled water at specified temperatures and then the plants were returned to the greenhouse bench.

Hot air therapy was investigated only with snapdragon rust. Because of the low heat capacity of the incubators the introduction of potted plants markedly lowered the incubator temperatures, and an hour or so usually elapsed before the incubators reached their original desired temperature. Also, the incubator temperature only was measured, and the leaf temperatures would be less. For these reasons, the data on hot air therapy are considered less reliable than those of hot water therapy.

RESULTS

Dry air. Results of two trials in the greenhouse of the eradicant action of dry air for bean rust are presented in figure 1, A, and show values ranging from 100 per cent eradication in 2 hr. after inoculation to 0 per cent in 18 hrs. In two trials at 13° C. with sunflower rust, dry air gave complete eradication in 2 hr., 55 per cent in 6 hr., 25 per cent in 12 hr., and 0 per cent in 18 hrs. These values must be considered in relation to later results with fungicides, as they indicate that chemical treatments showed eradicant action long after dry air was ineffective.

Ordinary spray applications. Most spray applications are designed to cover the plant surface but not to penetrate the tissues. Such surface sprays, however, may give eradication of rust long after the fungus has developed beyond the stage where it can be eradicated by dry air. Results with 2 per cent rosin lime sulphur (8) on sunflower rust indicate that 100 per cent eradication was secured up to 16 hr. after inoculation, and that control decreased progressively to 0 at 90 hr. after inoculation (Fig. 1, A). This spray was therefore partially effective up to 77 hr. after dry air had no eradicant action. Other materials tested and results of a typical test 14 hr. after inoculation were: sulphur dust—5 per cent eradication, 1 per cent Bordeaux plus spreader—35 per cent eradication, 2 per cent lime sulphur plus spreader—86 per cent eradication, and 0.2 per cent copper resinate in emulsifiable pine oil—96 per cent eradication.

When plants treated in this manner were placed in a moist chamber after spraying, and the drying of the spray thus delayed, the eradication was more complete than when the plants were dried immediately after treatment. For example, in tests with sunflower rust at 18 hr. after inoculation, 2 per cent rosin lime sulphur gave 86 per cent eradication when the treated plants were dried immediately, and 100 per cent eradication when the plants were held in a moist chamber for 6 hr. after treatment. Unless otherwise mentioned, plants treated with eradicant sprays were held in a moist chamber for about 6 hr. after treatment.

When a spreader was added to any of the therapy sprays used, greater rust control resulted, presumably because the spreader facilitated penetration of the spray through the stomatal openings and into the intercellular spaces, even though the penetration did not reach the point where watersoaking of the tissues was apparent to the unaided eye. For example, 0.03 per cent copper sulphate without spreader gave 94 and 32 per cent eradication at 18 and 65 hr., respectively, but with a spreader gave 98 and
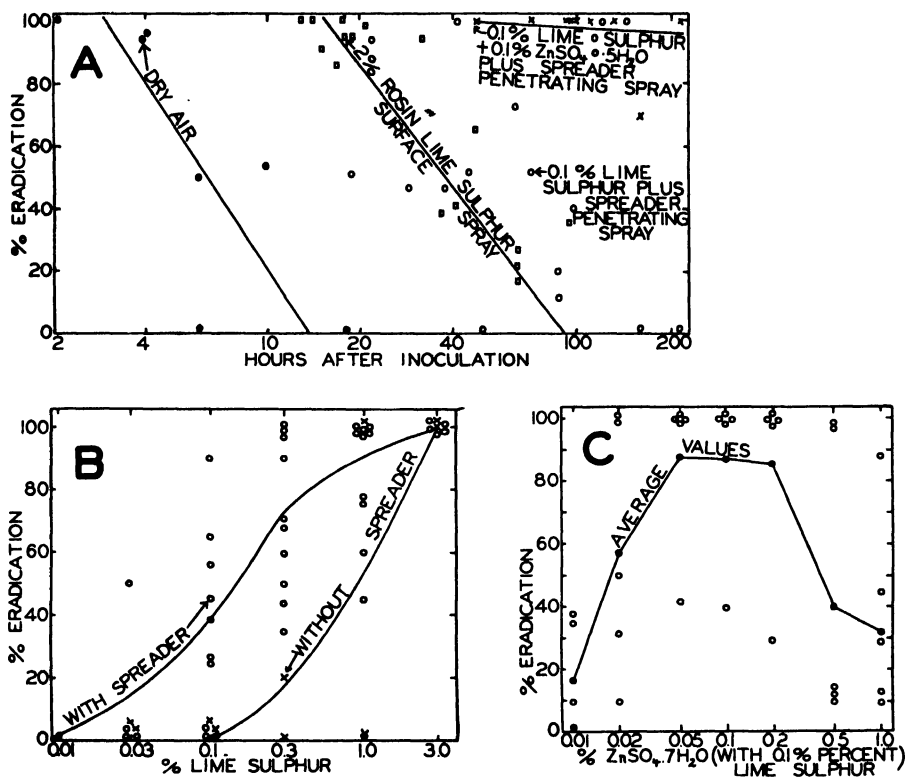
FIG. 1. A. Eradicant action of dry air for bean rust, rosin lime sulphur surface spray for sunflower rust, and lime sulphur and lime sulphur-zinc sulphate as penetrating sprays for bean rust. B. Effect of 0.05 per cent spreader on the eradicant action of various dosages of lime sulphur as penetrating sprays. C. Effect of varying dosages of zinc sulphate on the eradicant action of 0.1 per cent lime sulphur for bean rust.

74 per cent eradication at these same periods. A series of 8 trials with 0.1 per cent copper sulphate plus spreader followed by 6 hr. in the moist chamber over the period from 16 to 96 hr. after inoculation gave an average time-eradication response curve which could not be distinguished from that of rosin lime sulphur.

Penetrating spray applications. While ordinary spray applications on beans leave only a surface deposit, complete water soaking of leaves is readily attained by sprays, and is favored by high as compared to low impact pressures, morning application as compared to afternoon or night

applications, young leaves as compared to old leaves, and the use of a spreader as compared to the use of sprays without a spreader. Water soaking spray applications caused greater injury than surface applications, but injury was less when the treated plants were placed in a moist chamber until after dark, and then returned to the greenhouse bench, than when allowed to dry in the sun immediately after spraying.

Copper sulphate, lime sulphur, and mixtures of lime sulphur and zinc sulphate were most studied. Of these, copper sulphate was poorest, and applications of 0.01 to 0.1 per cent gave 70 to 90 per cent eradication of bean rust at 40 to 114 hr. after inoculation. Dosages of 0.01 to 3.0 per cent lime sulphur with spreader (Fig. 1, B) gave eradication values which were approximately those of a normal dosage response curve. Lime sulphur without spreader (Fig. 1, B) was less effective than lime sulphur plus spreader except at the 3 per cent concentration, which gave complete eradication in all tests.

Results of trials of the effect of age of infection on eradication of bean rust with 0.1 per cent lime sulphur plus spreader are given in figure 1, A. Eradication from sprays applied 9 to 215 hr. after inoculation ranged from 0 to 100 per cent with no clear relationship to time. This concentration is near the critical lower dosage for eradication (Fig. 1, B), and it appears that some inadequately controlled factor was important in determining these variable results.

The effect of addition of zinc sulphate to lime sulphur is presented in figures 1, A and 1, C. In figure 1, A, it is shown that 0.1 per cent lime sulphur plus 0.1 per cent zinc sulphate plus spreader gave 100 per cent eradication when applied 48 to 215 hr. after inoculation in 7 out of 8 trials, and was therefore more effective and more consistent than lime sulphur without zinc sulphate, an observation also supported by trials of this mixture as a protective spray (10). The effect of varying the concentration of zinc sulphate from 0.01 to 1 per cent is shown in figure 1, C, and it is clear that there exists a broad optimum between 0.02 and 0.5 per cent zinc sulphate.

Cy nide gas. Results with cyanide gas, for plants which were not killed by the treatment, are given in figure 2, A. In 2 trials with snapdragon rust, 80 to 100 per cent eradication resulted on 4-day-old infections exposed to calculated doses of 60 to 100 p.p.m. cyanide gas for 12 hr. in large cans. In 3 trials with bean rust in large cans, 0 to 100 per cent eradication of 4-day-old lesions resulted from 12-hour exposures to 60 to 400 p.p.m. In two trials with bean rust in 450-cc. jars, 70 to 100 per cent eradication of 4-day-old lesions resulted from 15-min. exposures to 600 to 2,000 p p.m. of cyanide. Eradication was generally poorer along the veins of the leaves, presumably because the leaves are thicker there. Usually there was some host injury from cyanide gas, but the greater susceptibility of the rust fungus than the host to cyanide gas was marked in all cases

where the host was not killed, and in a few cases complete rust eradication resulted without apparent host injury. .

Volatile gases from lime sulphur and sodium sulphide. With exposure periods of 5 hours, the gases from dilute lime sulphur usually gave com-
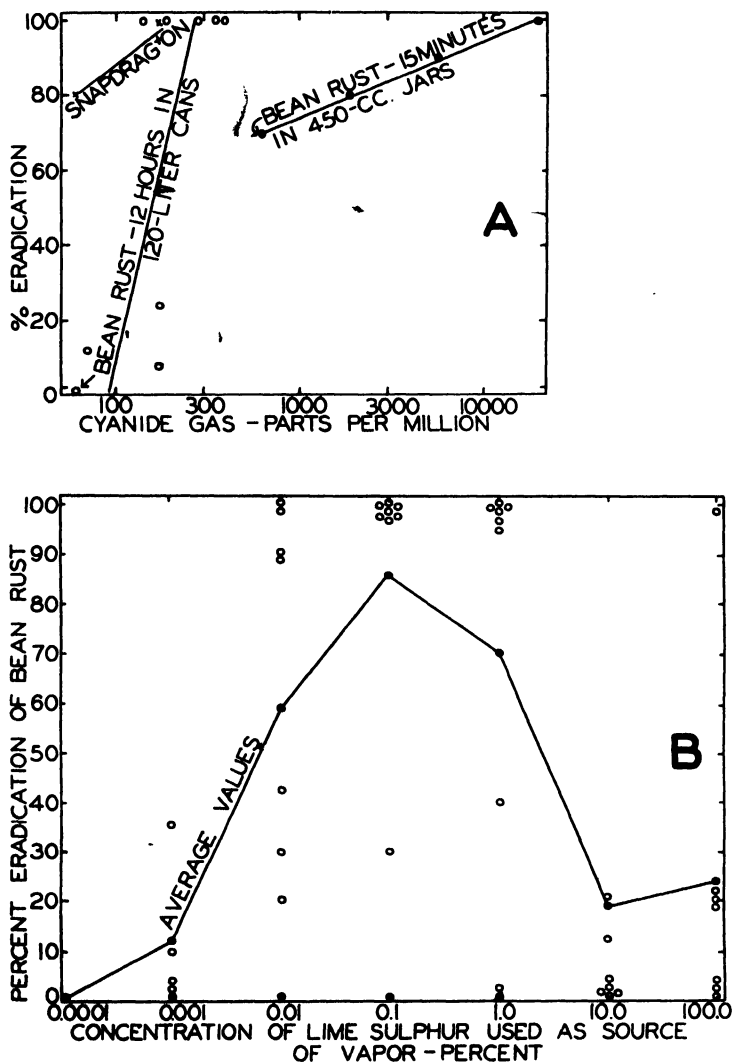
FIG. 2. A. Eradicant action of cyanide gas for snapdragon rust and bean rust. The tests on snapdragon rust, upper left, were exposures of 12 hours in 120-liter cans. B. Eradicant action on bean rust of the volatile products from various concentrations of lime sulphur.

plete eradication of 3- to 7-day-old bean rust infections without host injury in a large number of trials (Fig. 2, B). For commercial lime sulphur (specific gravity 1.28) diluted with distilled water the most therapeutic concentration was about 0.1 per cent, with less therapy resulting from lower or higher concentrations. The low therapeutic value of vapors from

high concentrations of lime sulphur is associated with their strong alkalinity, since wi.h 0.1 per cent lime sulphur and 0.05 per cent sodium sulphide, the optimum pH (as manipulated with 0.1 N HCl) for therapy
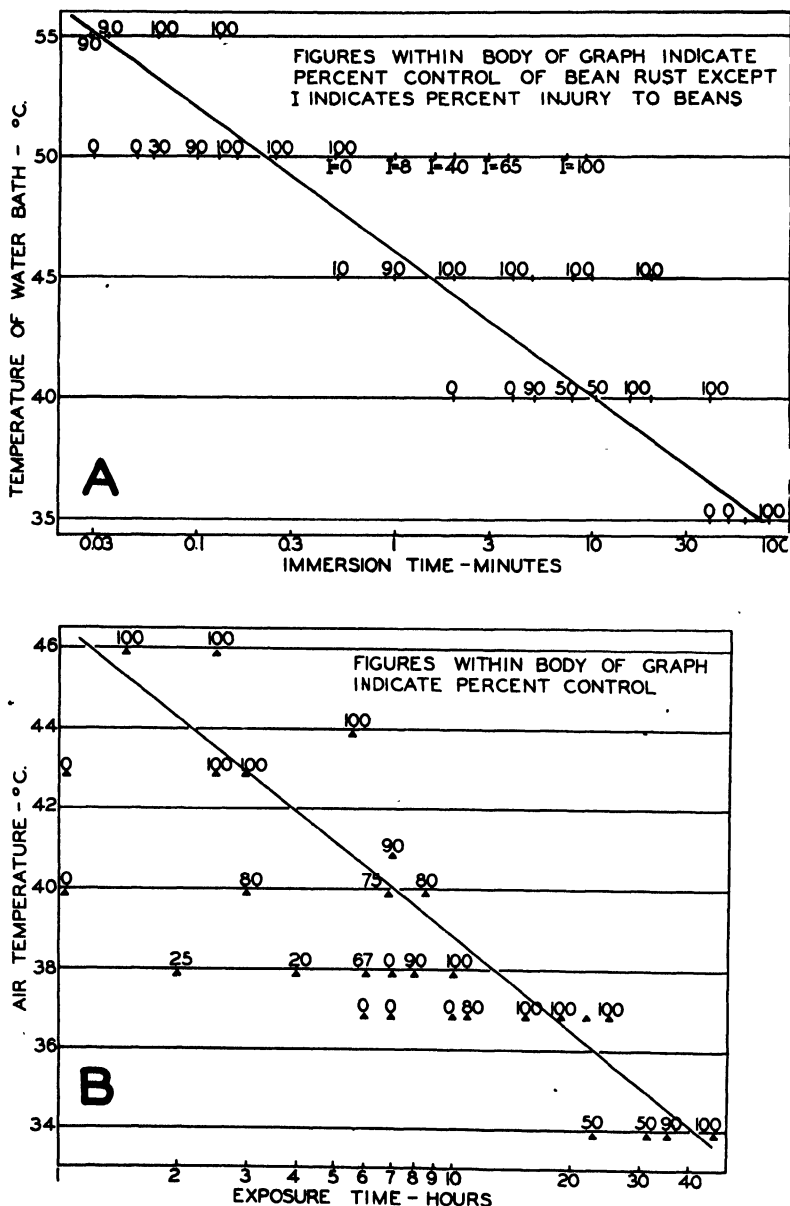
FIG. 3. A. Eradicant action of hot water for bean rust, and injury to beans from water at 50° C. B. Eradicant action of hot air for snapdragon rust.

without host injury was about pH 9, while the unadjusted pH of all solutions of these or higher concentrations was always above this. As judged

by the darkening of lead acetate paper in the test chambers, there was a correlation between hydrogen sulphide formed and therapeutic activity. However, in other trials with bean powdery mildew (*Erysiphe polygoni*) therapy resulted from a mixture containing 0.1 per cent lime sulphur + 0.1 per cent $KMnO_4$, where H_2S could usually not be detected with lead acetate.

Hot water. Results for bean rust of all therapy tests with hot water from 35° C. to 55° C., in 5°-intervals, are presented in figure 3, A. The results indicate complete eradication of bean rust in 80 min. at 35° C., 15 min. at 40°, 2 min. at 45°, 0.13 min. at 50°, and 0.06 min. at 55°. The best straight line through these points indicates a Q10 (ratio of immersion times for equivalent lethal effect over a range of 10° C.) of about 44 between 35° and 55° C. Uredial pustules killed by hot water made no further growth. If therapy treatments were made at 4 days after inoculation, when the rust pustules were barely apparent, no host injury usually occurred at the therapy dosages indicated, but at 7 and 10 days after inoculation, treatments which killed the rust usually killed the leaf tissue for about a millimeter beyond the pustule. As judged by results of one test with beans at 50° C., there was apparently a wide margin between eradication of the parasite and injury to the host (Fig. 3, A). There was no host injury from 30 sec. at 50° C., which was about 3 times the time necessary for complete eradication. For exposures of 30 sec. to 9 min. at 50° C. there was a progressive increase in host injury from 0 to 100 per cent.

In the one test of hot water therapy for mint rust, treatments of 10-day-old infections gave the following results: 10 min. at 40° C.—30 per cent eradication, 1.5 min. at 45° C.—80 per cent eradication, 12 sec. at 50° C.—100 per cent eradication, and 4 sec. at 55° C.—100 per cent eradication; all with no host injury. From these results it would appear that the relative sensitivity of mint rust and mint plants was probably not greatly different from that of bean rust and bean plants. There was no possibility in these experiments of the cure of mint rust being due to killing of the surface spores, as was the case in Niederhauser's (4) tests. In the successful treatments the killed uredial pustules were sunken, dull in color, and obviously inactive 10 days after treatment, while untreated pustules were raised, brown, and powdery.

Hot air. Satisfactory hot air therapy was secured only with snapdragon rust, and results of these tests, presented in figure 3, B, indicate that snapdragon rust can be eradicated by hot air dosages ranging from 30 min. at 46° C. to 40 hr. at 34° C., with a Q10 between these limits of about 20.

DISCUSSION

Therapy is only one of six basic methods of plant disease control, and is at present the least used. The frequent excursions of plant pathologists into this field of study have yielded relatively little of practical value for

many years, and the present study is no exception. However, the need for curative treatments exists, and undoubtedly practical uses will arise from basic studies of the subject. In the present study the practical value of hot water treatment to control rust infections in planting stock, as with mint rust, is demonstrated, and the possible control of rust diseases in greenhouses, by sulphur vapors, is indicated. The use of sulphur vapor in this way should also prove of value in further basic studies, such as studies of the relative absorption and fixation of sulphur vapors by healthy and fungus infected plant tissues.

The rust fungi studied are obviously more sensitive to the treatments used than are their hosts. With hot water the lethal exposure for bean rust at 50° C. was only about one-sixth that of the lethal exposure for bean leaves. This would indicate that bean rust has evolved under lower maximum temperatures than have beans. Evolutionary significance is less apparent in the results with sulphur and cyanide.

In other reports of heat therapy, specific times at a specified temperature are usually indicated. This study would indicate there is no optimum temperature, and that a great range of temperatures could be used (in the case of bean rust, from 35° to 55° C.) provided appropriate exposure times were chosen.

SUMMARY

Bean and sunflower plants inoculated with uredospores of their respective rusts, placed in a moist chamber, and returned to a dry atmosphere at varying times after inoculation, showed no subsequent infection when the plan s were dr.ed 2 hr. after inoculation, but increasing infection with incubation periods up to about 18 hr. before drying. When infected plants were sprayed with surface applications of 2 per cent rosin lime sulphur or 0.1 per cent copper sulphate plus spre::der, subsequent infection was inversely proportional to the interval between inoculation and spraying for per ods between 18 and 90 hr. after inoculation. Penetrating applications of dilute lime sulphur were effective in eradicating bean rust up to 8 days after inoculation with little host injury, and the effectiveness of these penetrating spray applications was increased with increasing lime sulphur concentrations, with the addition of a spreader or zinc sulphate to the lime sulphur spray, and with enclosure in a moist chamber following spraying.

Cyanide gas at concentrations ranging from 70 to 2,000 p.p.m. was effective in eradicating bean and snapdragon rust in exposures of 15 min. and 12 hr., but host injury usually resulted. The vapors from lime sulphur solutions ranging from 0.001 per cent to 100 per cent lime sulphur eradica ed bean rust from 4- and 7-day-old infections, with the maximum therapy from 0.1 per cent lime sulphur. The optimum alkalinity for the volatile therapeutic action of 0.1 per cent lime sulphur or 0.05 per cent Na_2S was about pH 9.

Four- to 10-day-old infections of bean rust and 10-day-old infections of
mint rust were killed without marked host injury when infected leaves
were immersed in water at 35° to 55° C. for intervals varying from about
80 min. to 4 sec., respectively, with a temperature coefficient of about 44.
Snapdragon rust was similarly eradicated with dry air at 34° to 46° C. in
40 hr. to 30 min., respectively, with a temperature coefficient of about 20.

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RESULTS OF ELEVEN YEARS' SPRAYING FOR PECAN SCAB CONTROL WITH HIGH-LIME AND LOW-LIME BORDEAUX MIXTURE

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INTRODUCTION

Pecan scab, caused by *Cladosporium effusum* (Wint.) Demaree, is one of the most important limiting factors in nut production, especially in areas of high rainfall and humidity, and its control is of vital importance to the pecan industry. All widely planted commercial varieties, other than the Stuart, are susceptible to scab in some localities and may require spraying to control the scab disease. The Stuart is the most widely planted of all varieties throughout the pecan-growing area.

Demaree and Cole (6) found that strains of the scab fungus show selective parasitism for certain varieties, and for that reason "trees of susceptible varieties might be growing in the same orchard in contiguous rows, or even with their branches interlocking those of scab diseased trees of another variety and not be affected with the disease. Varieties are sometimes very susceptible in one locality and apparently immune in another."

Observations by the writer indicate that the build-up and increase of virulent strains of the fungus from susceptible varieties to those previously resistant is a gradual process and usually takes place over a period of several years.

EXPERIMENTS TO CONTROL PECAN SCAB

Early investigators, Waite (13), Spooner (12), McMurran and Demaree (8), Neal (9), Demaree and Cole (4, 5), Nolen (10), and Boyd (2), did considerable experimental work on the control of pecan scab using the standard spray and dust materials, i.e., high-lime Bordeaux mixture, lime-sulfur solutions, and copper-lime dusts. In addition, the recommended schedules stipulated that the first application be postponed until after the nuts had been pollinated. Most spraying recommendations included the use of two spray guns for each tree—one to spray lower and one upper portions of the tree simultaneously. When the trees were sprayed according to these recommendations there was usually an accumulation of Bordeaux mixture on the leaves, therefore spray or drought injury occurred in most orchards during late summer and early fall, especially during seasons when the daily distribution of rainfall was less than normal. Certain growers considered spraying so hazardous that it was practically abandoned.

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No injury from fungicides applied as dusts was noticeable but commercial control was not obtained, therefore dusting was likewise abandoned.

Baker and Cole (1) sprayed pecan trees with 4–6–100 Bordeaux mixture before the pistillate flowers were pollinated and no injury to the flowers or foliage occurred, provided the temperature was above 55° F. It had earlier been considered hazardous by most research workers to spray in the prepollination stage because of the injury that might result.

Demaree and Large (7) reported moderate spray or drought injury to Schley pecan trees following 4 applications of 4–5–100 Bordeaux and severe injury when 4 applications of 6–8–100 and 6–16–100 Bordeaux were used. The extent of the injury depended upon the distribution of rainfall as well as the variety of pecans sprayed. The Schley variety was highly susceptible to drought or spray injury. All spray applications were made after the pistillate flowers had passed the receptive stages, and two spray guns were used.

Wilson and Runnels (14) report that Ginseng was severely injured when sprayed with 3 applications of 6–9–100 Bordeaux mixture in Ohio during the summer of 1930, while the seasonal distribution of rainfall was less than normal. In the final stages of this injury the sprayed plants collapsed and died, as if suffering for want of moisture. The writers did not describe the type of spray equipment used to apply this spray. Neither did they state the dosage of Bordeaux mixture applied.

In 1935 Cole and Large (3), while testing various fungicides, successfully controlled scab on the very susceptible Schley variety by using only one spray gun and making one prepollination application of 4–1–100 Bordeaux mixture, followed by 3 applications of 6–2–100 Bordeaux mixture. No visible injury resulted to the leaves or nuts.

Following the preliminary successes obtained in 1935, 36 Schley trees were randomized in single tree plots of 9 trees each to accommodate 4 treatments. The trees were given 3 applications of one of the following sprays in 1936: 6–2–100 Bordeaux mixture, 6–2–100 Bordeaux plus 1 quart summer oil emulsion, or 6–6–100 Bordeaux. Checks were not sprayed. These treatments followed one prepollination spray application of 4–1–100 Bordeaux mixture used uniformly on all sprayed plots. The various Bordeaux mixtures were compared in relation to drought or spray injury on pecan foliage. These plots were sprayed according to this schedule each succeeding year for eleven years.

Only one spray gun was used to apply the material and the operator always stood on top of the spraying machine. Each tree was circled in applying the spray and the machine was never stopped. The sprayer used had a pump capacity of 35 gal. per min. and was operated by its own power. The trees were 30 years old when the test was begun and the average amount of material used was 25 gal. per application and approximately 100 gal. per season.

Table 1 contains a summary of the yield of nuts in pounds and the number of orchard-run, cured nuts required to weigh a pound.[2]

The yield from trees sprayed with the high-lime (6–6–100) formula did not differ significantly from the yields from trees sprayed with the low-lime (6–2–100) formula.

All yields from sprayed trees were highly significantly greater than the yields from the unsprayed check trees.

The differences in the number of nuts per pound between the three spray treatments were not significant. However, the difference in weight

TABLE 1.—*The 11-year mean yields of Schley*[a] *pecan nuts in pounds per tree, and the number of nuts per pound, as influenced by the application of various Bordeaux mixtures*

Treatment	No. of trees per treatment	Mean yield, lb. per tree	Mean No. nuts per lb.
1 application of 4–1–100 Bordeaux mixture followed by 3 applications of 6–2–100 Bordeaux mixture	9	50	73
1 application of 4–1–100 Bordeaux mixture followed by 3 applications of 6–2–100 Bordeaux mixture plus summer oil emulsion, 1 qt.–100	9	45	70
1 application of 4–1–100 Bordeaux mixture followed by 3 applications of 6–6–100 Bordeaux mixture	9	42	72
Check, no spray	9	18	107
Difference required for significance at 5 per cent level		9	19
Difference required for significance at 1 per cent level		13	25

[a] Schley variety, ten trees per acre.

of the nuts from sprayed trees and from the check trees was significant at the one per cent level.

At no time during the course of this experiment was there any evidence of spray or drought injury to the trees in any of the treatments used in the test. Apparently the injury formerly reported as "spray" or "drought" injury was due to the method of application of the material which resulted in over-spraying and was not due to the Bordeaux mixture used.

The trees in the plots sprayed with 6–2–100 Bordeaux gave slightly higher yields of nuts than those in either the 6–2–100 Bordeaux plus summer oil emulsion or the 6–6–100 plots. Since the first formula is more economical than the other mixtures, the 6–2–100 Bordeaux mixture is recommended for pecan scab control.

SUMMARY

Results are given of spraying tests for eleven years with 6–2–100 Bor-

deaux mixture, a 6–2–100 Bordeaux plus summer oil emulsion, and a 6–6–100 Bordeaux to control pecan scab, only one spray gun being used so as to avoid over-spraying.

There was no significant difference in yield of nuts resulting from the various Bordeaux mixtures used. The differences in yield between the sprayed and the unsprayed trees were highly significant.

Since there was no visible sign of spray or drought injury on any of the sprayed trees used in this test, the writer believes the spray injury reported by former workers may be attributed to the method of application rather than to the materials used.

Because a 6–2–100 Bordeaux mixture is more economical and produces slightly higher yields of nuts than either a 6–2–100 Bordeaux plus oil emulsion or a 6–6–100 Bordeaux mixture, it is recommended for pecan scab control.

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ERGOT AND STERILITY IN BAHIA GRASS[1]

GLENN W. BURTON AND C. L. LEFEBVRE[2]

Ergot (*Claviceps paspali* Stevens and Hall) was observed on Bahia grass (*Paspalum notatum* Flugge) at Tifton, Georgia, for the first time in 1938 and has occurred in considerable abundance every year since. During these years most of the infected florets have been filled with ergot sclerotia and covered with sticky "honey-dew" exudate. Only a very few florets, however, have been found with sclerotia large enough to pry the lemma and palea apart and extend beyond them.

The direct association between ergot and sterility in Bahia grass was first observed in five Paraguay × common Bahia hybrids in 1941. These hybrids were highly sterile as indicated by their poor seed setting performance but were more heavily infected with ergot than either parent.

Other hybrids produced later gave rise to uniform progenies exactly like the female F_1 parent, indicating that they were reproducing by apomixis.[3] An analyses of seed samples taken from each plant within 40 plant progenies of these hybrids revealed that the hybrids differed significantly in the percentage of florets producing sclerotia.[3] A correlation coefficient of -0.86 was obtained between the average percentage of florets setting seed and the average percentage of florets producing sclerotia for these hybrid progenies, indicating that the percentage of sclerotia increased as the fertility of the hybrid progeny decreased. Since each of the hybrid progenies supplying the mean values for the correlation were different in appearance, they might have differed in ergot resistance, sterility, or both. Hence, it was impossible to ascertain whether the greater susceptibility to ergot prevented seed setting or the greater sterility allowed more sclerotia to be formed.

In 1941, a single male sterile plant was found growing in a progeny of 192 white stigma common Bahia plants. This individual differed from the other 191 plants only in its male sterility. Since the progeny was believed to have arisen by apomixis and the male sterile plant to have originated by mutation, it seemed highly probable that the gene complex of the male sterile was identical with that of the other 191 plants except for the gene or genes causing male sterility. The failure of F_1 hybrids between the male sterile and fertile plants to segregate made it impossible to ascertain the number of genes responsible for the male sterile mutant.[3]

The male sterile Bahia plant set no seed except when pollinated and

then produced seed giving rise to many apomictic plants like itself. When unpollinated heads of this clone were inoculated with ergot "honey dew" by smearing "honey dew" droplets on the florets, they became infected and produced as much "honey dew" as fertilized heads of its sister plants. This observation indicated that ergot development is not dependent upon the fertilization of the Bahia grass floret.

In 1942, twenty-seven male sterile plants and a similar number of fertile sister plants were spaced 3 feet apart so that a male sterile and a male fertile plant occurred together in pairs. Seed harvested from each of
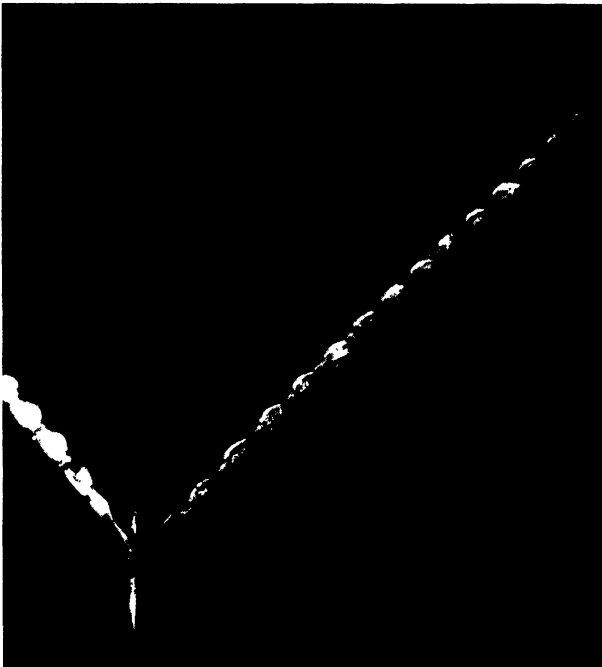
FIG. 1. Ergot "honey-dew" exuding from Bahia grass florets 8 days after artificial inoculation.

these plants were analyzed for the percentage of florets containing caryopses and the percentage of florets containing sclerotia. A summary of the results showed that the proportions of florets to set seed was 35.7 per cent for the male fertile plants and 6.2 per cent for the male sterile plants. Only 9.6 per cent of the male fertile florets contained sclerotia while 33.1 per cent of the male sterile florets contained sclerotia. These differences between the male fertile progenies exceeded the 1 per cent level of significance for both measurements. Since the male sterile and male fertile plants should have been genetically the same except for the male sterility factor, the differences in the amount of ergot produced by each must have been due to their differences in male sterility. This observation suggests that care must be exercised in an ergot resistance breeding program lest

differences in ergot readings due to sterility differences be mistaken for genetic differences.

An explanation for the observed relationship between ergot and sterility in Bahia grass can only be suggested at this time. The stigmas of the self fertile common Bahia grass usually begin to dry up within 24 hours after pollination if fertilization has occurred. When not pollinated, these stigmas remain fresh for many days. Consequently, the relative abundance of fresh stigmas on Bahia grass panicles furnishes a good index of the degree of fertilization and the seed set that may be expected later. The male sterile Bahia, for example, always shows a much higher proportion of fresh stigmas than the male fertile Bahia. These observations suggested that delayed pollination might be causing the greater incidence of ergot in the male sterile Bahia grass.

To test the theory a number of male sterile and male fertile florets were brought into the laboratory on September 20, 1947. Florets on a number of panicles blooming the first day were painted white and those opening on the second day were painted pink. All florets not blooming by the third day were removed. All florets were heavily pollinated each day. Approximately 30 minutes after pollination on the third day all panicles were immersed in a water suspension of ergot "honey dew." Several unpollinated panicles of the male sterile Bahia were inoculated at the same time to check the efficacy of the technique. Eight days later "honey dew" was observed exuding from many of the florets on the inoculated unpollinated male sterile panicles. See figure 1. Only three sclerotia were found in more than 200 florets that were pollinated 30 minutes before inoculation. No sclerotia were found in the florets pollinated 24 and 48 hours before inoculation. These observations suggest that pollination soon rendered the florets impregnable to the attack of ergot.

DISCUSSION

The observations reported here would seem to justify the following working hypothesis, which may be used to guide future investigations. Ergot apparently is capable of attacking and growing upon susceptible Bahia grass florets that are sterile because of cytological abnormalities or lack of effective pollination. Pollination and fertilization seem to set up a mechanism which soon renders the susceptible floret resistant to later attack by ergot. Consequently, only those florets inoculated with ergot before or soon after effective pollination may become ergotized.

SUMMARY

1. Highly sterile Paraguay × common Bahia grass hybrids were much more heavily ergotized than either parent.

2. A correlation coefficient of − 0.86 was obtained between percentage seed set and ergot abundance in Bahia hybrid progenies varying significantly in fertility.

3. An apomictic progeny of a male sterile mutant produced over 5 times as many ergotized florets as a similar progeny of a male fertile sister plant compared with it.

4. Pollination and fertilization were shown to be unnecessary for the development of ergot in Bahia grass.

5. Susceptible Bahia grass florets inoculated and not pollinated produced many ergot sclerotia. Inoculating 30 minutes after pollination resulted in the production of a few sclerotia. Florets inoculated 24 and 48 hours after effective pollination developed no sclerotia. Effective pollination resulting in fertilization and seed set seems to soon render the florets of Bahia grass resistant to ergot attack.

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A NEW FUSARIUM DISEASE OF LANG (LATHYRUS SATIVUS)[1]

VISHNU P. BHIDE[2] AND B. N. UPPAL[3]

A destructive, new wilt disease of lang (*Lathyrus sativus* L.) appeared in the Broach district of Bombay Province, India, in 1935–36 and assumed serious proportions in subsequent years. Since a disease of this type had never been reported before, a study of it was undertaken with a view to describing its symptoms and determining the cause. The results of a part of the investigation are reported here.

SYMPTOMS OF THE DISEASE

Lang is an important cold weather crop in the Broach district of Bombay Province where 100,000 acres are grown annually. It is regularly cultivated in rotation with jowar (*Sorghum vulgare* L.) and cotton. It is an annual and is much prized for its green fodder; its grain is fed to cattle in times of scarcity besides its use as a pulse by the poorer classes. Losses from wilt are generally 10 to 15 per cent, though fields showing 60 to 70 per cent infection are not uncommon in some years.

The disease generally appears in late December when the plants are in full bloom. Patches of wilted plants appear at this time and these enlarge in all directions as the season advances.

Yellowing of leaves is the first noticeable symptom of wilt in the field; it starts with the oldest leaves and proceeds upwards until the whole plant is involved. Yellowed leaves become flaccid, droop, and eventually dry. Sterility of affected plants is very common, and if a few pods are already formed before a plant becomes infected, they never mature and contain only a few, shrunken seeds. Discoloration of the vascular system, characteristic of Fusarium wilts, is found only in the root system. Partial wilting is also a common symptom. When once infected, plants slowly dry up and die.

In pot-culture in the greenhouse, drooping of leaves is the only symptom of wilt in young plants, which die very suddenly. Yellowing of leaves is sometimes seen when older plants are infected. Vascular discoloration of the root system is present in such plants, whereas young infected plants seldom show it.

PATHOGENICITY ·

The fungus was very easily isolated from roots of infected plants by the usual method of planting infected tissue on potato-dextrose agar. Numerous isolations were made from wilted plants collected from various localities in the Broach district. In the majority of cases, the isolations yielded a species of *Fusarium* which did not produce any pigment or fructification

on potato-dextrose agar, but showed an abundance of single, ovoid micro-
conidia and smooth, single, terminal or intercalary chlamydospores. No
macroconidia were produced on this medium.

The pathogenicity of the fungus was determined by growing plants in
steamed river bottom soil that had been infested with a pure culture from
Richards' solution. The infested soil was held in a closed metal bin for four
weeks and then placed in clay pots disinfested in 5 per cent copper sulphate
solution. Four seeds were sown in each of twelve pots and held in the
greenhouse at 23° to 28° C. A comparable number of cultures employing
steamed, non-infested soil were held in the same greenhouse as a check.

Wilt appeared when seedlings were 12 days old, and progressed rapidly
until about 70 per cent of the plants had wilted in a month. No disease
appeared in the checks. Isolations from wilted plants yielded a typical
Fusarium which was identical with the original culture that had been
placed in the soil.

RELATION OF TEMPERATURE TO THE GROWTH OF THE FUNGUS

The temperature-growth relations of the fungus were studied on Richards'
agar. Plates of this medium were inoculated with a young culture of the

TABLE 1.—*Mean colony diameter after six days' growth on Richards' agar at dif-
ferent temperatures*

Temperature	Mean colony diameter	Temperature	Mean colony diameter
°C.	cm.	°C.	cm.
0.0	0.0	24.5	4.3
7.2	0.0	27.0	5.0
11.1	0.5	29.0	4.8
15.5	1.3	31.0	3.8
20.0	2.0	33.0	0.6
22.0	4.0	35.0	0.0

fungus and incubated at various temperatures. A spore suspension of the
fungus was used to inoculate the plates with a standard loop. Triplicate
plates were carried at each temperature and growth was measured as mean
colony diameter at the end of six days' incubation. Table 1 shows the
results.

The fungus has a wide growth-temperature range; the optimum is about
27° C., and the minimum and maximum about 11° and 33° C., respectively.

RELATION OF SOIL TEMPERATURE TO WILT

It is well known that soil temperature is a limiting factor for the develop-
ment of Fusarium wilts. The effect of soil temperature on wilt in lang was
determined by growing it in Fusarium-infested soil maintained at various
soil temperatures in Wisconsin soil temperature tanks.

In the first trial, four tanks were set up to maintain soil temperatures of
20°, 25°, 30°, and 35° C. The daily fluctuations in every case were not more

than ± 0.5° C. Each tank held six cans of infested soil and one of steamed
soil as a check. Twenty seeds of lang, previously disinfected in a 1 : 1,000
solution of mercuric chloride were then sown in each can. Temperatures,
both soil and air, were recorded three times a day.

Wilt appeared at 25° C. when the seedlings were seven days old and at
20° and 30° when they were 10 days old. The appearance of the disease
was delayed at 35° C. until the plants were 22 days old. Records of deaths
were taken every three days, and the test was stopped when no more plants
wilted. Each surviving plant was then carefully uprooted and its roots
were examined for vascular discoloration. Those plants with vascular
necrosis of the roots were classed as infected and the rest as healthy.

In another trial, five soil temperature tanks were set up to maintain soil
temperatures of 27°, 30°, 32°, 35°, and 37° C., respectively. Wilt appeared
when the seedlings were seven days old at 27° C. and when they were 12
days old at 30° and 32° C., whereas at 37° C. germination was very poor

TABLE 2.—*Percentage of plants with infection at different soil temperatures*

Mean soil temperature (°C.)	No. of plants	Percentage of wilting observed			Vascular necrosis (Per cent)
		After 10 days	After 20 days	After 32 days	
19.9	91	0.0	48.5	69.0	94.0
25.0	87	2.0	59.0	81.0	100.0
29.9	82	0.0	13.0	60.0	90.0
34.8	85	0.0	0.0	9.0	52.0
27.0	158	2.0	25.3	75.5	85.4
29.9	89	0.0	12.3	54.6	66.9
32.5	73	0.0	9.3	12.5	12.5
34.8	84	0.0	0.0	6.0	1.6
37.0	37	0.0	0.0	0.0	0.0

and the resulting plants remained stunted and unhealthy. No wilt appeared
at this temperature. The survivals were examined as before for root dis-
coloration. The results of these trials are given in table 2.

The results show that wilt reaches its maximum development at soil tem-
peratures between 25° and 27° C., at which level it is characterized by the
earliness of attack and the severity of infection. At lower and higher levels,
the incubation period increases and the severity decreases. These observa-
tions, taken together with the temperature-growth relations of the fungus
indicate that a positive correlation exists between the growth of the fungus
and disease development at various soil temperatures.

These results are similar to those obtained by Gilman (3), Tims (7),
and Tisdale (8) for *Fusarium conglutinans* on cabbage, Clayton (1) for
Fusarium lycopersici, causing wilt of tomatoes, and Uppal and Kulkarni (9)
for *Fusarium vasinfectum* on *Crotolaria juncea*.

HOST RANGE

Plants of several species were grown in soil infested with *Fusarium* from
lang to see whether they were susceptible to this parasite. About 50 seeds

of each of the following were sown in infested soil held at 25° C. in soil temperature tanks (the optimum for disease incidence in lang): sweet peas (*Lathyrus odoratus*), everlasting peas (*Lathyrus latifolius*), cabbage (*Brassica oleracea*), celery (*Apium graveolens*), beet (*Beta vulgaris*), garden peas (*Pisum sativum*), field peas (*Pisum sativum* var. *arvense*), gram (*Cicer arietinum*), and cotton (*Gossypium herbaceum*). Lang was sown as check.

After one month, about 80 per cent of the lang was dead but all plants of the other species remained healthy. At the end of two months, every plant was uprooted and the roots were examined for discoloration, which was absent in all cases except lang. These results indicate that the fungus is restricted in its parasitism to its own host, as far as the species tested are concerned.

MORPHOLOGY AND CULTURAL CHARACTERS OF THE FUNGUS

Growth on various media. The fungus was grown on a number of media in order to obtain a complete range of spore forms as recommended by Wollenweber et al. (10). Synthetic agar, potato hard agar, and Richards' agar were also used as done by Doidge (2). The cultures were grown at room temperature (24° – 27° C.) in darkness and observations were made regularly. Ridgway's (5) color nomenclature was used to describe the colors produced by the fungus.

No sporodochia, pionnotes, or sclerotia were produced on any media. Microconidia and chlamydospores were produced on all media whereas pigment was evident only on a few media. The results are summarized in table 3.

TABLE 3.—*Growth characteristics of the fungus on different media*

Medium	Mycelium (aerial)	Pigment		Macro-conidia
		Mycelium	Substrate	
Green bean pod	moderate	pale yellow green	none	few
Banana peel	sparse	pale yellow orange	none	few
Steamed rice	moderate	white	faint violet	none
Potato hard agar	moderate	white	none	none
Synthetic agar	profuse	white	pale orange	none
Richards' agar	profuse	white	pale orange yellow	none
Oatmeal agar	thin	white	cinnamon-brown	none
Potato-dextrose agar (2 per cent)	moderate	white	none	none
Potato-dextrose agar (5 per cent)	none	none	none	none

Measurements of conidia and chlamydospores.

  Conidia: from three-weeks-old culture on bean pod.
      0-septate, 70.5 per cent, 7.4 (3.4–10.6) × 3.4 (2.5–5.0) microns.
      1-septate, 20.0 per cent, 15.8 (12.5–17.5) × 4.4 (3.8–5.0) microns.
      2-septate, 4.0 per cent, 21.6 (18.8–25.0) × 3.8 (3.0–5.0) microns.
      3-septate, 5.5 per cent, 24.3 (17.5–32.5) × 3.3 (3.0–3.7) microns.
  Conidia: from three-weeks-old culture on banana peel.
      0-septate, 92.0 per cent, (not measured).
      1-septate, 4.0 per cent, (not measured).
      2-septate, rare, (not measured).
      3-septate, 4.0 per cent, 30.3 (25.0–42.5) × 4.3 (3.8–5.0) microns.
  Chlamydospores: from one-month-old culture on 2 per cent potato-
      dextrose agar.
      9.9 (7.9–15.4) microns in diameter.

Microconidia are single, hyaline, ovoid or ellipsoid. Macroconidia are straight or slightly curved, non-pedicellate, hyaline, and with delicate walls and septation. Chlamydospores are single, terminal as well as intercalary, produced by the mycelium, rarely in conidia, spherical, smooth, and rich in contents. Conidiophores simple. Sclerotia absent.

IDENTITY OF THE PATHOGEN

The presence of terminal and intercalary chlamydospores, delicate walls and septation of the macroconidia, and the abundance of single, one celled, ovoid microconidia place the *Fusarium* in the section Elegans; within this section, the total absence of sporodochia immediately identifies the fungus with the sub-section Orthocera. For a species diagnosis of the fungus, the characters of value are the absence of pionnotes, non-erumpent stroma, simple conidiophores, color on steamed rice, and the size of 3-septate conidia. These characters align the fungus with *Fusarium conglutinans* Wr. which it closely resembles except that it is non-pathogenic to cabbage and produces a faint violet color on steamed rice.

If color production is considered a major criterion for diagnosis of species in this sub-section, the fungus then resembles *F. orthoceras* App. and Wr. The pigment produced is not, however, as intense as that of *F. orthoceras*.

Padwick (4) made a detailed study of 11 of the 12 Orthocera-Fusaria using authentic cultures and came to the conclusion that there was no basic difference in the two species, *Fusarium conglutinans* and *F. orthoceras*. These two differ mainly in color production and size of conidia besides pathogenicity, but Padwick (4) showed that these two characters are extremely variable. In his experiments, *F. conglutinans* and all its varieties were non-pigmented whereas *F. orthoceras* and its varieties could be classified into three groups based on color production. A purple color was produced by *F. orthoceras* and its varieties *apii* and *longius*, a blue color was produced by *F. orthoceras* var. *pisi*, and no pigment was produced by *F. orthoceras* var. *apii* f. 1. Moreover, the colors produced were not necessarily the same as those described by Wollenweber and Reinking (11).

As regards spore size, the non-septate conidia of any of the species showed variations which were as great as or greater than those between species. As most of the species did not produce any macroconidia, variations in this character could not be measured.

Snyder and Hansen (6) in 1940 formally recommended the merging of all the species, varieties, and forms of Elegans-Fusaria (41 in all) into one single species, *Fusarium oxysporum* Schlect. In order to achieve this, they emended the description of *F. oxysporum*. The various parasitic members of the section Elegans became biologic forms of the master species, and the saprophytic forms were merged with it. They presented experimental evidence to show that the characters that go to differentiate between the subgroups of the section Elegans (presence or absence of sporodochia and the size of spores) are not stable and hence the absence of justification for maintaining them. The authors believe that there is no need for such a drastic step since it normally is possible to place an Elegans Fusarium in its proper sub-group following the key of Wollenweber and Reinking (11). Moreover, since pathogenicity remains a criterion for delimitation of pathogenic forms, the proposed change does not improve things a great deal.

In the present work, the variations in the size of 3-septate conidia is very striking. On bean pod the spores measure 24.3 (17.5–32.5) × 3.3 (3.0–3.7) μ, whereas on banana peel the measurements are 30.3 (25.0–42.5) × 4.3 (3.8–5.0) μ. In the key of Wollenweber and Reinking (11) for the Orthocera-Fusaria, the average measurements of 3-septate conidia for *Fusarium conglutinans* and *F. orthoceras* are 34 × 3.5 μ and 33 × 3.5 μ respectively, a difference that is not significant. The only difference in the two species seems to be the absence of color in *F. conglutinans*, and yet a variety of *F. orthoceras* is non-pigmented. The authors agree with Padwick in his decision to unite the two species as there is no real difference between them. It is therefore proposed to describe the lang pathogen as a new variety of *F. orthoceras* with the varietal name *lathyri* on account of its pathogenicity to *Lathyrus sativus*. A technical description of the fungus follows:

Fusarium orthoceras App. and Wr. var. **lathyri** n. var.

Stroma pale, colorless on most media, cinnamon-brown on oatmeal agar, faint violet on steamed rice, non-erumpent. Aerial mycelium colorless, sometimes sending out ochraceous strands. Sporodochia, pionnotes, and sclerotia absent. Microconidia scattered freely in the mycelium, single, ovoid to ellipsoid, measuring on an average 7.4 (3.4–10.6) × 3.4 (2.5–5.0) microns and constitute about 90 per cent of the conidia produced. Macroconidia rarely produced, mostly 3-septate, straight or slightly curved, with delicate walls and septation, non-pedicellate, and measure on an average 27.3 (17.5–42.5) × 4.0 (3.0–5.0) microns. Conidiophores simple. Chlamydospores numerous, terminal and intercalary, single, smooth, spherical, measuring on an average 9.9 (7.9–15.4) microns in diameter.

This fungus causes a vascular wilt of *Lathyrus sativus* L. in the Broach district of Bombay Province, India.

Stromate pallido; sporodochiis, pionnote et sclerotiis deficientibus; micro-conidiis numerosis, instratis, continuis, ovoideis, 7.4×3.4 $(3.4–10.6 \times 2.5–5.0)$ μ numerosis; macroconidiis raris, 3-septatis orthoceris vel leniter fal-catis, non-pedicellatis, 27.3×4.0 $(17.5–42.5 \times 3.0–5.0)$ μ; chlamydosporis nu-merosis, terminalibus et intercalaribus, singulis, globosis, 9.9 $(7.9–15.4)$ μ diam.

Habitat: In disco trunci *Lathyri sativi* L. Bombay, India.

SUMMARY

Wilt in lang (*Lathyrus sativus* L.) is a destructive disease in the Broach district of Bombay Province, India. A new *Fusarium,* isolated from wilted plants, proved pathogenic to lang grown in soil infested with pure cultures of the fungus.

Yellowing of leaves is the first noticeable symptom of wilt in the field. Sterility of affected plants is also common. Discoloration of the vascular system is found in the roots only.

The fungus has a very wide growth-temperature range of $11°–33°$ C., the optimum being about $27°$ C.

The pathogen is most destructive at soil temperatures between $25°$ and $27°$ C. At lower and higher levels, the incubation period lengthens and the severity decreases.

In experiments on host range, no infection was obtained on sweet peas, everlasting peas, garden peas, field peas, cabbage, celery, beets, gram (*Cicer arietinum*), and cotton (*Gossypium herbaceum*).

The fungus compares favorably with *Fusarium conglutinans* Wr. but differs from it in the presence of color and its inability to infect cabbage. The fungus is made a new variety of *F. orthoceras* App. and Wr. because of its pigmented character and the varietal name *lathyri* is proposed for it on account of its pathogenicity to *Lathyrus sativus.*

A technical description of the fungus is given.

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REPORT OF THE 1948 ANNUAL MEETING OF THE
SOUTHERN DIVISION, THE AMERICAN
PHYTOPATHOLOGICAL SOCIETY

The 1948 Annual Meeting of the Southern Division of The American Phytopathological Society was held in part as a section at the meeting of the Association of Southern Agricultural Workers February 12, 13, 14, in Washington, D. C. A joint session was held on February 12 with the Potomac Division at Beltsville, Md. About sixty Southern plant pathologists were present and over twenty formal papers were presented.

A business session was held on the morning of February 13, when the following officers were elected:

President, I. L. FORBES
Vice-President, L. M. BLANK
Secretary-Treasurer, J. L. LYLE

Titles and abstracts of papers presented follow.

I. L. FORBES, *Secretary-Treasurer*

Varietal Reaction of Oats to Helminthosporium victoriae. ATKINS, J. G. and E. R. STAMPER. Helminthosporium or Victoria blight of oats was first identified in Louisiana on oats grown from introduced seed in the spring of 1947 and caused severe losses in the 1947 crop. Several of the varieties grown in Louisiana have been tested for varietal resistance in field and greenhouse. Artificially infested seed were used in the field test while a suspension of inoculum was sprinkled over seed planted in infested soil in the greenhouse tests. The percentage of seedlings showing the disease was used in evaluating resistance. In the field test Clinton and Nortex were highly resistant; Fulghum, La. 42–48, Ferguson 922, Alber, Appler, and Camellia were resistant; and Traveler, Letoria, Quincy Red, and Victorgrain were highly susceptible. On the basis of greenhouse tests the same general trends were obtained as in the field test. In addition, Benton, Hastings 100 bushel, Nortex 107, and a number of Coker's Hybrids were highly resistant; Lee Rustler, Ranger, and Louisiana Red Rustproof were resistant; and DeSota, Fultex, Lega, Stanton, Vicland, and Fulgrain were susceptible. Seed treatments have not given favorable results; some of the mercurials have drastically reduced stands when used in excess. Preliminary studies indicate that the fungus is found more frequently on or within the glumes than on the seed itself.

Control of Sweet Potato Scurf by Vine Cuttings. COOK, HAROLD T. Badly scurfed sweet potatoes were treated with borax and bedded in sand. The sprouts were pulled from half of the bed and vine cuttings (sprouts cut approximately 1 inch above the sand) were taken from the other half. Five two-row (285 ft. long) replications of sprouts and cuttings were alternated in a field that had been in sweet potatoes as recently as 1945. Data were taken at harvest time. Yield of primes per row: sprouts, 187 lb.; cuttings, 234 lb. Percentage of scurf free primes: sprouts, 78; cuttings, 97. Scurf severity index: sprouts, 36.7; cuttings, 6.1. Percentage of plants with scurfed underground stems: sprouts, 53.7; cuttings, 0.2. The results indicate that scurf infected sprouts were the most important source of scurf and that scurf free potatoes may be obtained by planting vine cuttings.

Anthracnose of Blue Lupine Is Seed-borne. DECKER, PHARES. Anthracnose (*Glomerella cingulata*) was reported from Florida in the fall of 1943 and 1946 as attacking lupine seedlings. During the spring of 1947 anthracnose was common in western Florida and southern Alabama, where it caused a material reduction of the seed crop. Stems, leaves, and seed pods were attacked and many seeds failed to develop. Seed lots from the combine contained from 4 to 10 per cent infected seeds after passing through the cleaner. Seed that bore acervuli filled with spores were taken from the warehouse in October and planted in the greenhouse. An average of 40 per cent of the infected seed produced seedling plants of which 75 per cent developed normal seedlings while 25 per cent bore cotyledons but failed to develop true leaves. Typical anthracnose lesions ap-

peared at or above the cotyledon attachment and on the leaves in 21 days. In a few days numerous acervuli filled with spores appeared in the lesions. None of the ten chemical seed-treating materials tested in the greenhouse prevented seedling infections from the seed-borne inoculum.

Breeding of Eggplants for Resistance to Phomopsis Blight. DECKER, PHARES. In 1940 two strains of eggplants, Pegan and Bengan, from India, were reported to be resistant to *Phomopsis verans* (Sacc. & Cyd.) Harter. These strains produce green to yellow fruit and bear large spines on the stems, leaves, and calyxes. No infected plants of these strains have been observed in the field, but successful inoculations have been made in the greenhouse. Crosses with the commercial varieties have produced disease-resistant hybrids without spines and with purple colored fruit. Fruit of the majority of the F_1 plants had the purple color of the commercial varieties, but a range of fruit colors, green to purple, was found in the F_2 and first backcross generations. Selections from the progenies through the F_6 and fifth backcrosses do not produce uniformly purple-colored fruit and disease resistance is largely lost by the fifth backcross. Crosses between selected hybrids appear to offer some promise in fixing a true-breeding, disease-resistant, purple fruited variety of eggplant adapted to Southern conditions.

Buckeye Rot-Resistant Tomatoes. FELIX, E. L. Fruit of the following 11 tomatoes proved highly resistant to buckeye rot, caused by *Phytophthora parasitica-terrestris,* in natural and artificial inoculations at the Tennessee Agricultural Experiment Station: Yellow Pear; Fargo Yellow Pear; U. S. Regional Vegetable Breeding Laboratory Nos. T244–0–1–4, T244–5–3, T244–10–3, T244–2–10–2, T7–013–5–52 (P.I. 79532 selection), T510–51 (P.I. 126923–51 selection), T994–51 (P.I. 126934 selection), T6–02–M6; and apparent segregates of Sherbakoff's 57 × Oxheart. Resistant fruits range from about $\frac{1}{2}$ to 2 inches in maximum diameter; from round to oblong, pyriform, and papillate; from moderately thin- to very thick-skinned; and they are either red or yellow. Sherbakoff's cross (No. 57 × Oxheart) from the standpoint of fruit size and resistance to buckeye rot, as well as resistance to Fusarium wilt, appears to be the most promising for further development. Sherbakoff's 57 is a highly wilt-resistant red segregate from F_2 seed of Tucker's 9–608–9 (Earliana × currant) × Break O'Day × Bison × Bison × Break O'Day.

Preliminary Studies in the Control of Strawberry Fruit Rots by Fungicides. FELIX, E. L. Seven fungicides were applied to strawberries in 1 to 4 applications, at 5 to 7-day intervals, beginning 1 week after blossom appearance and ending 1 week before harvest began, in a series of 95 small plots, protected against drift, at Knoxville, Tennessee. They were copper carbonate 13 and 0.42 per cent, Phygon (2,3-dichloro-1,4-naphthoquinone) 4 and 0.12 per cent, Fermate (ferric dimethyldithiocarbamate) 7.5 and 0.24 per cent, and Mycoban (sodium propionate) 16 and 0.5 per cent, respectively, as dusts and sprays; Tribasic copper sulphate 14 per cent and Spergon (tetrachloro-parabenzoquinone) 7.5 per cent as dusts; and Dithane D-14 (disodium ethylene bisdithiocarbamate hexylhydrate) 0.75 per cent spray. All sprays, except Phygon wettable, contained 0.00125 per cent Triton 1956B as wetting agent. Rates of application were 40 lb. of dust and 150 gal. of spray per acre. Not any of the fungicides, as tested, appear promising for strawberry fruit rot control, except possibly copper carbonate against Botrytis gray mold, which comprised only about 12 per cent of the total rot. None seemed to affect yield, although Dithane, Phygon, copper carbonate, and Tribasic copper sulphate resulted in slight foliage injury. Dithane, even in a single early application, decidedly impaired the flavor of fresh strawberries harvested throughout the season.

Interaction of Nitrogen Fertilization and Powdery Mildew on Yield of Wheat. HEBERT, T. T., W. H. RANKIN, and G. K. MIDDLETON. Nitrogen topdressing of wheat in early spring is recommended in North Carolina. Since this practice increases the amount of powdery mildew on wheat, it became of interest to determine how this increase in mildew affects the yield increases obtained from the use of nitrogen. In two experiments nitrogen was applied at different times on plots where mildew was controlled by dusting with sulfur and on undusted plots in a split-plot design. The experiments were carried out on sandy loam soil, relatively low in nitrogen, and to which an adequate supply of phosphorus, potassium, calcium, and magesium had been added.

Since the results were similar for the two experiments, only one will be discussed in detail. A highly significant interaction was obtained between nitrogen fertilization and mildew control. Dusting had very little effect on yield where no nitrogen was applied or when 45 lb. nitrogen was applied at seeding time (Oct. 24); but when this amount of nitrogen was applied as a topdressing in the spring, the dusted plots yielded as much as 40 percent more than the undusted plots. The Feburary 1 application of nitrogen gave a significantly higher yield than the March 1 application in the undusted series, but

fell slightly below it in the dusted series. Also, the use of nitrogen at seeding time produced as high a yield as its application in March in the undusted series, but gave definitely inferior results in the dusted series. Thus the presence of mildew on wheat may affect both the optimum time for application of nitrogen and the response to nitrogen.

A Crown Rot of Alfalfa Caused by Colletotrichum trifolii. HENDERSON, R. G., and T. J. SMITH. During the summer and early fall of 1947 dying alfalfa plants were observed in a number of fields. An examination of these plants revealed a stem and root rot progressing downward from the crown. The diseased tissue was yellowish-brown to reddish-brown. It appeared that the infection entered the crown through a shoot or stubble and then involved the entire crown and tap root. Isolations from the diseased tissue consistently yielded *Colletotrichum trifolii*. Lesions were on the shoots but were not conspicuous. Acervuli could usually be found, but in many instances sporulation was sparse. Although anthracnose crown rot, has not been identified in many alfalfa fields over the State, it was observed causing extensive damage at widely separated points. It is known that summer killing of alfalfa by unidentified diseases is of major importance in maintaining stands. The association of *C. trifolii* with this type of injury strongly suggests that anthracnose may be of greater importance on alfalfa than previously thought.

Relative Susceptibility of Alfalfa Varieties to Certain Foliage Diseases. HENDERSON, R. G. and T. J. SMITH. Severe defoliation of alfalfa occured in many fields in southwest Virginia in 1947. In July and August Stemphylium leaf spot (*Stemphylium botryosum* Wallroth) predominated, while in October and November Pseudopeziza leaf spot (*Pseudopeziza medicaginis* (Lib.) Sacc.) was the most important. There appeared to be a distinct difference in the amount of defoliation on different varieties. Plots of 13 varieties were examined carefully on July 3 and on October 2 and each plot scored according to the severity of the leaf diseases. A disease index on the basis of the maximum defoliation being scored 100 was then calculated for each variety on each date. On July 3, Ladak and Hardistan varieties had the greatest amount of diseased foliage and were given a disease index of 88, and 70, respectively. The Ranger variety was intermediate with a disease index of 60. The varieties with the lowest disease index were Williamsburg 23, Kansas Common 23, Buffalo 27, A–14 27, and Hardigan 31. On October 2, the scores for the several varieties followed the same trend as those obtained earlier. The varieties Ladak and Hardistan had the greatest amount of disease. Ranger was intermediate, and Williamsburg, Kansas Common, Buffalo, A–14 27, and Atlantic were the least affected. Pseudopeziza leaf spot and black stem (*Ascochyta imperfecta* Peck.) caused progressively greater defoliation on all varieties during October and November. Individual plants in certain varieties remained remarkably free of defoliation and suggested that more highly resistant strains could be selected out of the standard varieties.

Soil Fumigation for Cigar-Wrapper Tobacco in Florida. KINCAID, RANDALL R. Soil fumigation tests were conducted at the North Florida Experiment Station in 1947, using dichloropropene-dichloropropane mixture at the rate of 20 gal. per acre and ethylene dibromide (10 per cent weight) at 30 gal. per acre. Applications were made in January, nearly 3 months before transplanting. The treated tobacco crop showed the following results, as compared with an untreated crop: Substantial reduction in root knot and coarse root (nematode root rot), increases in yield ranging from 200 to 400 lb. per acre, and no important differences in grade and fire-holding capacity of the leaves.

Results from Dusting Soybeans with Copper in 1947. LEHMAN, S. G. and J. H. GRAHAM. Three varieties of soybeans, Ralsoy, Ogden, and Roanoke, were dusted six times at approximately 8-day intervals with a mixture· consisting of tribasic copper sulphate equivalent to 7 per cent metallic copper, 10 per cent wheat flour, 3 per cent DDT, and 80 per cent Cherokee Clay. Control plots received the same dust devoid of copper. Only light to moderate damage from bacterial diseases occurred during the summer; as the plants reached the peak of the vegetative period, severe damage occurred on leaves from a disease not fully identified as to cause. Disease scores calculated on October 1, five weeks after the final dust application, showed 27.6 per cent of diseased leaf area on control plots. On plots receiving 30, 60, and 90 lb. copper dust per acre at each application, diseased leaf area was reduced to 19, 12, and 11 per cent, respectively. Mean yield of the three varieties on the control plots was 27.0 bushels per acre. Plots receiving 30, 60, and 90 lb. copper dust yielded 32.5, 32.5, and 32.7 bushels, respectively. A yield increase of 5.5 bushels per acre resulted from use of copper dust. Least increase required for significance at the one per cent level was 5.1 bushels.

Soybean Seed Treatment Tests in North Carolina in 1947. LEHMAN, S. G. and J. H. GRAHAM. After preliminary germination tests of treated and untreated soybean seed in steamed sand in the greenhouse, ten lots were selected for field testing. These included paired lots grown from the same parental seed in widely separated areas, McCullers and Plymouth, where weather conditions differed markedly during the ripening and harvesting period. Three aliquots of each lot were planted, one untreated, one dusted with Arasan, (tetramethyl thiuram disulfide), one with Spergon (tetrachloro parabenzoquinone). Three plantings, early, medium, and late were made. The mean increase from Arasan was 41, 80, and 8 per cent, respectively. Emergence of nine lots was increased and diseased seedlings decreased more by Arasan than by Spergon. Seed produced at McCullers gave high emergence and few diseased seedlings while seed from Plymouth gave relatively low emergence and many diseased seedlings in both field and greenhouse plantings. Emergence was negatively correlated with seed borne infection. Emergence from both untreated and treated seed was lower in the field than in the greenhouse. However, the difference from treated seed was only about one-half that from untreated seed. Field performance of seed lots can be more accurately predicted from preliminary greenhouse germination tests if treated seed are used and proper appraisal is made of seed infection.

A Special Method of Isolating Thielavia basicola from Tobacco Roots. LUCAS, G. B. The ascomycete *Thielavia basicola* is found frequently on tobacco roots infected with the black root rot fungus *Thielaviopsis basicola*. Repeated attempts to isolate the *Thielavia* resulted in failure even though numerous media and techniques were tried. However, if roots containing perithecia of *Thielavia* are washed in tap water and incubated at 37° C. on Richard's agar with filter paper as a carbon source (instead of sucrose) the fungus can be isolated from a high percentage of the roots.

The Occurrence of Texas Root Rot of Cotton in Louisiana in 1947. NEAL, D. C. *Phymatotrichum omnivorum* root rot of cotton was found in Louisiana for the first time on August 12 in Bossier Parish, the location being on Highway 10 about four miles north of Bossier City and east of Red River. Approximately 30 acres of land, embracing two separate fields, are infested at this location, and, in addition to cotton, the disease also was found attacking carrots, beans, and turnips. In a survey conducted with the Parish Agents of Caddo and Bossier Parishes from August 26 to 28, the disease also was located on August 26 near Dixie, Louisiana, in Caddo Parish about 18 miles north of Shreveport and 2 miles west of Highway 71 close to the west bank of Red River. The infested land at Dixie comprises approximately 4.5 acres, with cotton and sweet potatoes affected. Considering the acreage involved, the damage to crops at both locations is severe, the mortality in cotton being about 85 per cent and the truck crops only slightly less. All of the other Red River alluvial—neutral to alkaline soils—of the State, including the Parishes of Pointe Coupee, Avoyelles, Rapides, Grant, Natchitoches, Red River, and the southern portions of Caddo and Bossier, were found to be free of root rot, but in some of these parishes cotton rust (potash hunger) was widespread and causing high losses. With respect to root rot, it appears that the disease on the two properties in Caddo and Bossier Parishes has been present for many years and may have been mistaken for cotton rust.

The Effect of Calcium and Other Ions on the Early Development of the Radicle of Cotton Seedlings. PRESLEY, JOHN T. and O. A. LEONARD. Considerable difficulty has been experienced in growing cotton seedlings with healthy radicles for laboratory experiments. The unhealthy condition of the radicles was at first considered to be the result of bacterial breakdown, but when every precaution was taken to maintain aseptic conditions the breakdown still occurred. In an effort to determine the cause of the breakdown, experiments were performed in which the effects of moisture tension during the germination period, aeration, temperature, and the concentration and kind of ions added to the solutions were studied. Seedlings that germinated under high moisture tension were especially susceptible to breakdown. Aeration or temperature had no definite effect upon the breakdown, but elongation of the radicles was markedly affected by temperature. The concentration and kind of ions added to the solutions had a pronounced effect upon the development and health of the radicles. Calcium salts, 0.001 to 0.004 M, added to the water in which the seedlings were placed resulted in a high percentage of healthy radicles. Salts of magesium, sodium, or potassium, 0.001 to 0.004 M, added to the water, singly or in combination, appreciably hastened the breakdown of radicles. The two conditions necessary for producing cotton seedlings with healthy radicles are adequate moisture during the germination period and adequate calcium during the early stages of growth.

Bacterial Canker of Cowpeas in Oklahoma. PRESTON, DUDLEY. The most devastating disease of cowpeas in Oklahoma is bacterial canker, caused by *Xanthomonas vignicola* Burkh. This disease occurs throughout the cowpea growing area of the south. The pathogen is carried within the seed, and is spread in the field by moisture propelled by wind. Chemical seed treatments tested gave no control. Possibility of control was definitely linked with the use of resistant varieties, such as Brabham, New Era, Groit, Iron, Victor, and others. Chinese Red is the most susceptible variety under Oklahoma conditions.

Helminthosporium carbonum in Virginia. ROANE, C. W. and C. F. GENTER. *Helminthosporium carbonum* race 1 was observed on corn in Virginia for the first time in 1947. Seed of inbred K44 obtained from Indiana was planted in an isolated block with K41 to make the single cross K44 × K41. Diseased K44 plants were first observed in middle August and by September all K44 plants had been attacked and were turning brown. The ears at harvest were severely damaged. All K41 plants remained green throughout the season. In an inbred nursery 1.5 miles away, infected ears of lines C.I.5, Ky.35–7, and P–8 were found. No further evidence of the disease in the field was observed, but greenhouse tests show that seedlings of many other inbred lines may be infected with the disease.

Breeding Wheat for Resistance to Leaf and Stem Rusts in Tennessee. SHERBAKOFF, C. D. The breeding was initiated in 1937. In 1938, rust-resistant wheats—Ceres × H–44, Hope × Hussar, Kenya, Minturki, and Supreza, and 8 rust-susceptible varieties—Honor, Jones Winter Fife, Leaps, Oro × Turkey/Florence, Shepherd, and 3 local ones were crossed in all possible combinations. The F_1 seedlings were vernalized and, in 1939, the F_2 plants which looked most promising just before making the time for making crosses were used to make 17 double- and 54 back-crosses to 13 soft wheats. In 1944, some of the rust-resistant segregates were backcrossed to 10 soft wheats—Yorkwin, Currell, Thorne, Trumbull, Hardired 5, and 5 local selections. The segregates were first judged by the field performance of single plants in head rows, in blocks of single-plant progenies, then in rod rows, and finally in standard yield-plot tests. The results to the present time have only begun to show practical benefits, since a number of the new wheats have just reached formal yield-test and increase stages. The greatest number of promising segregates came from crosses of Supreza × Hope-Hussar by Honor and by Head 129.

Spraying and Dusting for Control of Onion Mildew in Louisiana. TIMS, E. C. Mildew is very destructive in the State on onions grown for seed as well as those grown for bulbs. However, the disease does not develop in epidemic proportions every year. For the past four years (1944–1947 inclusive) some control tests have been conducted for onion mildew. The following spray materials were used in one or more tests: Bordeaux mixture alone and combined with DDT (dichlorodiphenyl trichloroethane), Spergon (tetra chloro benzoquinone, wettable), Fermate (ferric dimethyl dithiocarbamate 70 per cent), Puratized N5D (phenyl mecuri triethanol ammonium lactate), Tribasic copper, Lime-sulphur, Zerlate (70 per cent zinc dimethyl dithiocarbamate), Dithane D-14 (active ingredients disodium ethylene bisdithiocarbamate 25 per cent, inert 75 per cent), COCS, and Phygon (technical 23 Dichloro-1-4 naphthoquinone—98 per cent, inert 2 per cent). One copper dust was used that contained 7 per cent metallic copper and 2 per cent mineral oil in Bentonite. None of these materials gave any effective control of mildew on bulb onions, but in one test Bordeaux mixture reduced the amount of mildew in seed onions. The copper dust also showed some promise on seed onions in the 1947 tests.

A New Fusarium Wilt of Sumac. TOOLE, E. R., W. C. SNYDER, and G. H. HEPTING. A new vascular wilt of staghorn sumac (*Rhus typhina*) was discovered on the Blue Ridge Parkway, near Waynesboro, Virginia, in 1946. Numerous clumps in a 5-acre field were dead and dying, and the disease appeared to be of recent origin. Further scouting in 1947 disclosed the fact that the wilt occurred for a distance of about 10 miles along the Parkway in this area. The disease is characterized by wilting and sometimes yellowing of the foliage, followed by death of the wilted plants. The vascular tissue of roots and stems has a brown discoloration typical of Fusarium wilts. A form of *Fusarium oxysporum* was consistently isolated from affected plants. Thirteen 1-month-old seedlings were inoculated in the greenhouse. All of them wilted within 2 months, and the fungus was reisolated from the vascular tissue of all of them. Check plants remained healthy.

Effects of Inoculations with a Fusarium on Gum Flow from Naval Stores Pines. TRUE, R. P., and A. G. SNOW, JR. Isolates of *Fusarium lateritium* from pitch cankers on *Pinus caribaea* prolonged gum flow from *P. caribaea* (slash), and *P. palustris* (longleaf). The rate of flow from untreated wounds, made in commercial turpentining

operations, is reduced considerably after 5 days. With *Fusarium* inoculations made ,in the spring, gum flow was maintained without appreciable reduction for 3 to 4 weeks for longleaf and 6 to 8 weeks for slash. Reinoculations made at different seasons and at different distances above the first wounds have failed to maintain the original gum flow rate. Inoculations made later in the season on additional trees were partially successful only on slash pine.

Soil Treatment Control of Fusarium Wilt and Nematodes of Cotton. SMITH, A. L. Two-year studies with Dowfume W–10 (10 per cent ethylene dibromide by volume) indicate practically complete control of wilt and nematodes was obtained by applying 30 to 37 gal. of this material per acre. The acre yield of Coker 4 in 1–7 cotton was increased from 367 to 1067 lb. and of Deltapine 14 cotton from 64 to 904 lb. of lint in a test on Catawba fine sandy loam, deep phase, in 1947. This amounts to 191 per cent and 1312 per cent increase for treatment, respectively. The percentage of wilt was reduced from 52.7 to 1.8 and from 96.8 to 3.2 for the same varieties. Coker 4 in 1–7 is comparable to most available wilt-resistant commercial varieties, while Deltapine is rather susceptible. A second test on six varieties in 1947 with the Dowfume applied at the rate of 12.5 gal. per acre in the rows gave slightly less control of wilt and nematodes. A highly susceptible variety, Hurley's Rowden, was not adequately protected. Miller 610, Dixie Triumph 366, Cook 142, and Coker 4 in 1–7 with varying amounts of wilt resistance, gave yields comparable to those obtained from the heavier rates of application.

REPORT OF THE FIFTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held on February 11 and 12, 1948, at the U. S. Plant Industry Station, Beltsville, Maryland, with approximately 100 members and guests in attendance. Thirty research papers were presented. All of the papers in the afternoon session of February 12 were given by visiting members of the Southern Division. This was followed by a dinner and an illustrated talk on "Agricultural Potentialities in Alaska" by Dr. Olaf Aamodt.

Officers elected for 1948–49 are as follows: *President*, W. F. JEFFERS; *Vice-President*, C. L. LEFEBVRE; *Secretary-Treasurer*, J. B. DEMAREE; *Councilor*, PAUL R. MILLER.

WILLIAM W. DIEHL, *Secretary-Treasurer*

ABSTRACTS OF PAPERS

Association of Gibberella zeae and Helminthosporium sativum as Related to the Development of Wheat Headblight. ANDERSON, A. L. Conidia of *Helminthosporium sativum* germinated only 50 per cent, with short knobby germ tubes, when mixed with *Gibberella zeae*. This reaction occurred only when high concentrations of spores (at least 10^6 spores per ml. of water) were used; normal germination occurred at lower concentrations of *G. zeae*. Furthermore, *G. zeae* colonies inhibited linear growth of *H. sativum* in culture. No inhibition of seed infection by *H. sativum* on wheat heads resulted from inoculum consisting of equal mixtures of *G. zeae* and *H. sativum*; thus at 24°–25° C. an equal number of seeds became infected with each fungus after the application of mixed inoculum containing 10^5 or 10^6 spores of each pathogen. When inoculum consisted of many spores of one pathogen plus a few of the other, infections by the latter were suppressed. For instance, inoculum consisting of 10^6 spores of *H. sativum* and 10^5 spores of *G. zeae* resulted in only 8.9 per cent of the seed becoming infected with *G. zeae* as compared to 32.2 per cent with 10^5 spores of *G. zeae* alone. That *G. zeae* did not preclude *H. sativum* seed infection is due probably to the lower number of spores per unit area of host tissue as compared to the concentrations used in the germination studies.

The effect of temperature and relative humidity upon the viability of the conidia of Piricularia oryzae. ANDERSON, A. L., B. W. HENRY, and T. L. MORGAN. The conidia of *Piricularia oryzae* retained viability best under cool, dry conditions in storage experiments in which the relative humidities were controlled with sulfuric acid solutions. Approximately 75 per cent of the conidia were viable after 12 months storage at 8° C. and 20 per cent relative humidity. At higher relative humidities (45, 70, and 95 per cent) the loss in viability was rapid at all temperatures. For example, after 5 months in storage, viable conidia were present only in those samples stored at 8° C. (all relative humidities) and at 20 per cent relative humidity at the other temperatures (20°, 28°, 32° C.). The number of lesions produced per plant by conidia stored for a 5 month period varied inversely with the temperature and the relative humidity during storage, being greater on those plants inoculated with conidia stored under cool, dry conditions. These tests also indicated that the percentage germination of the conidia was not a satisfactory method for predicting spore infectivity since conidia stored at 8° C. and 95 per cent relative humidity, having then 50 per cent viable cells, were unable to cause appreciable infection.

Two Additional Mosaic Diseases of Iris. BRIERLEY, PHILIP, and FLOYD E. SMITH. The common mosaic of bearded iris is readily transmitted to *Belamcanda chinensis* by leaf-rubbing and by *Myzus persicae*, producing a well-defined systemic mottling. The efficiency of transfer makes this plant useful for indexing bearded iris. The previously described mosaic of bulbous iris, transmissible by the same means, does not infect *Belamcanda*, nor does the bean yellow mosaic from gladiolus. A more virulent mosaic from *Iris spuria* and *I. aurea*, both of the section Apogon, is transmissible to *Belamcanda* by leaf-rubbing and by *Myzus persicae*, killing this test plant consistently in about 30 days. This virus is readily superimposed on previously established non-lethal bearded

iris mosaic in *Belamcanda*, with the same fatal effect. The three iris viruses and bean yellow mosaic are transmissible to bulbous iris seedlings by leaf-rubbing or by *Myzus persicae*, inducing current-season symptoms from early inoculations, and can be reisolated in suitable test plants. Only the bearded iris mosaic has been transmitted to bearded iris, and only the bean yellow mosaic to *Vicia faba*. All except the *Iris spuria* mosaic are infectious to gladiolus seedlings. Properties of all three iris viruses have not been determined satisfactorily, but appear to be similar, as are the vector relations of these viruses.

Natural Infection of Solanum dolcamara by Phytophthora infestans. Cox, CARROLL E. *Solanum dulcamara* L. naturally infected by *Phytophthora infestans* was found in Maryland on July 10, 1947. The diseased plant was growing on a fence around a garden in which tomatoes and potatoes were also affected by the late blight fungus. The pathogen was sporulating in foliage lesions on all three suscepts, but less abundantly on *S. dulcamara* than on the other two. Sporangia and sporangiophores from the three suscepts appeared identical microscopically. Inoculation of detached, healthy leaves in the laboratory showed that sporangia from each of the three suscepts were equally capable of producing infection of tomato, *S. dulcamara*, petunia, and pepper, and all failed to cause infection of *S. carolinense* L. and tobacco. The appearance of lesions and the degree of sporulation on the various leaves inoculated were the same regardless of source of inoculum. The pathogen naturally infecting these three suscepts was, therefore, believed to be the same. The possibility exists that *S. dulcamara* may function as an overwintering suscept for *P. infestans* in Maryland.

Organic Fungicides in the Control of Certain Shade and Ornamental Tree Diseases. DAVIS, SPENCER H. Studies were conducted at The Morris Arboretum at Philadelphia, and in Delaware, using Bordeaux 8–8–100, Zerlate (zinc dimethyldithiocarbamate) 1½–100, Fermate (ferric dimethyldithiocarbamate) 1½–100, Parzate (zinc ethylene bisdithiocarbamate) 1½–100, Bioquin 1 (copper-8-quinolinolate) 1–100, and Puratized (phenyl mercuri triethanol ammonium lactate) 1 pint–100. Leaf blotch (*Guignardia aesculi*) of horsechestnut and buckeye was controlled with Bordeaux, Zerlate, and Parzate, while Fermate and Puratized gave only slight control. Zerlate, Parzate, and Puratized controlled leaf spot (*Gnomonia ovata*) of hickory; Fermate failed. Fermate controlled anthracnose (*Gleosporium fructigenum*) on sassafras. Bioquin 1 and Parzate gave excellent control of leaf blight (*Entomosporium maculatum*) of pear. Leaf blight (*Entomosporium thuemenii*) of English hawthorn was controlled by Bordeaux, while Puratized gave control only until mid-summer. No twig blight (*Sphaeropsis sp.*) was present in red oak treated with Bordeaux or Puratized. Parzate only slightly controlled anthracnose (*Gleosporium apocryptum*) of maple. Twig blight (*Myxosporium sp.*) of dogwood was not controlled by any treatment. Control of tip blight (*Sphaeropsis ellisii*) of Austrian pine was erratic with all treatments. Puratized failed to burn-out quince rust (*Gymnosporangium clavipes*) on cedar. The only injuries were: Bordeaux caused necrotic spotting of leaves and petioles on horsechestnut, English hawthorn, and red oak; Puratized caused leaf fall of sour gum (*Nyssa sylvatica*) and retarded fall coloration of sour gum and dogwood.

A Gall Disease of Blueberry Caused by a Bacterium. DEMAREE, J. B., and NATHAN R. SMITH. Galls resembling those caused by the crown gall organism (*Agrobacterium tumefaciens*) on woody plants have been reported on blueberries from Michigan, New Jersey, New York, and Oregon. Occasionally the disease becomes serious in blueberry nurseries. The galls most frequently develop on small lateral shoots near the ground. Sometimes the main stems become infested. A bacterium was isolated from galls collected in New Jersey and Oregon and has been determined provisionally as an *Agrobacterium*, probably a strain of *A. tumefaciens*, formerly called *Phytomonas tumefaciens*. This organism readily infected blueberry plants when inoculated in wounded young shoot tissues, and caused large rough galls. It caused moderate-size galls on tomato plants, and was slightly pathogenic in tobacco, ragweed, and cranberry, but failed to infect *Kalanchoe*, *Azalea*, strawberry, *Ipomoea*, and *Convolulus*. It was lethal to young aster plants.

Camellia Wilt and Root-rot. GILL, D. L. A wilt and root-rot disease of *Camellia japonica* L. has been frequently observed for the past 2 years, particularly in plants 1, 2, and 3 years from cuttings, growing in coldframes and beds. The first symptom noticed is a yellowing of the foliage followed by wilting and death of the plant. This is preceded by browning and rotting of the roots and in some cases discoloration of the base of the plants. Branches only may die when part of the root system is killed and new roots are formed. Losses range from 10 per cent to practically 100 per cent. Plants not killed show marked stunting. *Phytophthora cinnamomi* Rands has consistently been isolated from diseased plants. Inoculations resulting in infection and reisolation have been made.

This fungus has also been obtained from roots of old plants showing dying back of the branches and sudden wilting of the plant. The disease is favored by moist conditions. The varieties Pink Perfection, Prince (Eugene) Napoleon, Pope Pius IX, Hermes, and Imura are severely affected while Prof. (C. S.) Sargent and Debutante are much less affected. This disease has not been positively identified on any variety of Camellia sasanqua Thumb.

Yield Response of Vegetable Plants Sprayed with Dithiocarbamate Fungicides. HEUBERGER, J. W. Research data obtained over a five-year period in Delaware show that tomatoes, potatoes, cantaloupes, and cucumbers (for pickles) sprayed with zinc dithiocarbamate fungicides have produced higher yields than when sprayed with copper fungicides, even when disease control by the dithiocarbamates has been inferior. This increased yield response has been ascribed largely to the lower phytotoxicity of the dithiocarbamates. However, research on cucumbers during 1946–1947 on the effect of various fungicides on yield before the downy mildew disease appeared showed that the iron and zinc dithiocarbamate fungicides increased the yield approximately 25 per cent over that of untreated plants, that Bordeaux mixture increased the yield approximately 10 per cent, but that a fixed copper fungicide reduced the yield slightly in both years. Two theories have been advanced by others to explain the increased yield response obtained by the use of certain dithiocarbamate fungicides: (1) the stimulatory effect that is caused by the zinc that some of these fungicides contain, and (2) the utilization by the plant of the nitrogen that these fungicides contain. The writer advances the hypothesis that the carbon, and possibly the sulfur, that these fungicides contain may be the factor largely responsible for the increased yield response. This hypothesis is supported to some extent by the data presented on cucumbers.

A New Virus Disease of Blackberry. HORN, NORMAN L. A new virus disease of blackberry, *Rubus allegheniensis,* was observed in a raspberry growing district in Maryland. The symptoms consist of a strikingly variegated leaf pattern. The disease was found to be graft transmissible from blackberry to blackberry, from blackberry to black raspberry (hybrid Cumberland × Evans), and from black raspberry to blackberry. The symptom pattern is expressed similarly in both blackberry and raspberry, but there is considerable variation in the pattern expressed in leaflets of individual leaves of both plants. The variation ranges from leaflets having only a few white islands to leaflets which are nearly totally white.

Transmissibility of the Mild Streak Virus of Black Raspberry. HORN, NORMAN L. Of 45 healthy black raspberry plants grafted to mild streak infected black raspberries 34 (75 per cent) became infected. When dodder was used to test transmission, eight of 40 (20 per cent) plants became infected. Varieties used in these tests were Logan, Dundee, and a hybrid of Cumberland × Evans. Field observations indicate the possibility of several strains of the mild streak virus as evidenced by the variations in the degree of cane symptoms on the same variety. When healthy plants were grafted to field cuttings showing varying degrees of cane symptoms, the test plants which became infected showed no corresponding degree of symptom expression.

Effect of Fusarium Isolates on Two Gladiolus Varieties. McCLELLAN, W. D. Marked differences in pathogenicity occurred when twenty mass culture isolates of Fusarium from gladiolus including *Fusarium oxysporum* var. *gladioli* Massey and *F. orthoceras* var. *gladioli* McCulloch were used to inoculate two varieties of gladiolus, Picardy (formerly considered resistant) and Dr. F. E. Bennett (highly susceptible). Differences were expressed both in rapidity of foliage symptom appearance and in type of corm rot. Some isolates caused a vascular rot, some a surface rot, and others caused a combination of symptoms. The pathogenicity of isolates varied from year to year. Disease severity (measured by the number of days short of the normal maturing period) of one culture was 7.0 and 106.0 in Picardy and Dr. F. E. Bennett, respectively; of another, 39.3 and 96.8; of a third, 107.5 and 107.5; and of a fourth, 107.5 and 55.4. In general, all isolates were more pathogenic in sand than in soil. The disease severity of five single-spore isolates from one culture were 9.9 and 18.3, 50.0 and 56.9, 53.8 and 46.7, 29.9 and 42.6, and 18.1 and 9.0, in Picardy and Dr. F. E. Bennett, respectively. When a mixture of three single-spore isolates was reisolated from plants of six Iridaceous genera and used to inoculate the two gladiolus varieties, marked differences in pathogenicity occurred. No morphological differences between isolates were observed.

Studies on End Rot of Stored Maryland Golden Sweet Potatoes. SMOOT, J. J. and W. F. JEFFERS. Two of the fungi most frequently isolated from end rotted Maryland Golden sweet potatoes were found to be *Diaporthe batatatis* and *Fusarium oxysporum.* Pathogenicity tests at both high and uncontrolled humidities showed approximately 80 per cent infection for *Diaporthe* and 25 per cent for *Fusarium.* In tests of temperature

relationship to pathogenicity, the percentage infection was approximately the same at 50°, 60°, 70°, and 80° F.; but amount of rot increased with increased temperatures. The disease may be produced by either organism, or the combination of both. The symptoms of the *Fusarium* end rot were shriveled, hardened ends usually occurring on smaller potatoes. The *Diaporthe* produced a collapsed, darkened dry decay that affected all sizes of roots.

Inefficacy of Ethylene Chlorobromide as a Therapeutic Agent in the Treatment of Gardenias Infected with the Root-knot Nematode. TARJAN, A. C. In an attempt to determine the efficacy of ethylene chlorobromide as a control for gardenia root-knot, 27 two-year-old cuttings of *Gardenia jasminoides*, whose roots were severely infected with the root-knot nematode, *Heterodera marioni*, were subjected to various concentrations of the chemical in a water emulsion. At time of treatment, soil moisture was 7.01 per cent and soil temperature 67° F. The plants were divided into two groups: (A) Receiving only one treatment; and (B) receiving two treatments, two weeks apart. Method of application was as follows: 4 mutually equidistant holes were punched 2 in. deep into the soil around the perimeter within each of the 8-inch pots containing the plants. Each treatment of 0.40 cc., 0.25 cc., 0.10 cc., or 0.05 cc. was divided into 4 equal aliquots, injected into the holes and covered with soil. Each group was harvested one month after its final treatment and quantitative determinations of parasite population in the roots were obtained by a modification of the Baermann technique. It was found that the plants were capable of withstanding as much as one 0.25 cc. application of ethylene chlorobromide emulsion or two 0.10 cc. applications spaced two weeks apart. The only concentration showing any degree of control of the pathogen was one application of 0.25 cc. of the emulsion.

The Meadow Nematode Disease of Boxwood. TARJAN, A. C. During recent years, a virulent disease of boxwood has caused serious decline of this plant throughout a wide area in the eastern United States. Critical examination of the root system reveals isolated lesions or entire rootlets which appear brownish-black apparently as a result of attacks by the various meadow nematodes, *Pratylenchus spp.* Badly diseased roots, however, will not always yield the pathogen which is migratory in habit and evacuates dead and almost-dead roots. Continued attack by this nematode over a number of years results in proliferous lateral root formation above the points of attack with eventual formation of a shallow, densely-interwoven root system in the upper soil layers. Above ground symptoms may include defoliation; sudden death of branches, sometimes resulting in a "stag-head" appearance; a sickly stunted growth; and various types of foliage discoloration. The latter may include varying degrees of chlorosis, sometimes appearing as thin lateral or transverse streaks, but more often involving localized areas, especially at the leaf tip, which eventually become necrotic. Foliage of lightly infected plants may present a dark greenish-bronze appearance ranging to a light yellow-orange for badly infected plants. Symptoms of the disease are usually more pronounced during periods of drought or freezing temperatures.

Abstracts of papers presented by members of the Southern Division will be in the report of that Division. Other research papers presented at this meeting were as follows:

Soil Cover Prevents Wood Decay Under Basementless Houses. JESSE D. DILLER and THOMAS W. JONES.
Effect of Incorporating Technical DDT in the Soil on the Number, Green Weight, and Dry Weight of Blakemore Strawberry Plants. M. C. GOLDSWORTHY and J. C. DUNEGAN.
Progress of the Ring Rot Organism in the Host Plant. MATHILDE BENSAÚDE GOTZ.
Variation in Phytophthora palmivora. CARL HARTLEY.
A Case of Violet Scab on Pansy Seedlings. R. A. JEHLE and ANNA E. JENKINS.
Further Observations on a Leaf Spot and Blotch of Sorghum. C. L. LEFEBVRE and HELEN S. SHERWIN.
Peppermint Wilt and Problems in Breeding for Resistance. E. C. STEVENSON.
The Effects of D-D and Dowfume W-40 Soil Treatments on Nematode Control, Yield, and Quality of Tobacco. F. A. TODD.

PHYTOPATHOLOGICAL NOTES

Preserving Culture Media.—Tubed agar medium stored on a laboratory shelf or in a refrigerator dries and shrinks within a few weeks after it is made unless some method is employed to prevent evaporation. During the last two years a simple method of preserving tubed medium in fresh condition has been used at the Potato Investigations Laboratory, Hastings, Florida.

The medium was made, tubed, and plugged and the tubes placed in clean, one-quart, glass, fruit jars. Wide-mouth fruit jars proved better than narrow-mouth ones as test tubes can be placed in them in an upright position and they hold ⅓ more tubes than the latter. Test tube plugs were pressed down carefully before tops were placed on the jars, as any cotton left between the rim and top of a jar prevents sealing. The jars of tubes were then sterilized in a pressure cooker at 15 lb. pressure for 20 minutes. Of course, an autoclave should prove equally satisfactory.

Directions for cleaning and sterilizing jars in hot water before use, proper sealing of different types of jars, and the operation of a pressure cooker are given in pamphlets on home canning of vegetables and other foods which can be obtained at any store that sells pressure cookers.

Once a jar has been opened the cover should not be replaced over any unused tubes of medium. Unused tubes of medium left in jars whose tops were replaced immediately after some tubes were removed showed contamination 3 to 4 weeks later. It appears that sufficient moisture was trapped in the closed jars to enable *Trichoderma lignorum* (Tode) Harz. and other fungi to grow through the moist cotton plugs and into the medium in the tubes. Tubed medium left in open jars remained sterile.

Potato-dextrose agar and water blanks have been preserved by the method described. Other kinds of medium probably can be preserved in the same manner. The advantages of the method are obvious for if enough jars, test tubes, and other materials are provided, sufficient medium can be made and sterilized at one time to last a year or longer and it will always be in fresh condition for immediate use.—A. H. EDDINS, Potato Investigations Laboratory, Agricultural Experiment Station, University of Florida, Hastings, Florida.

Cactus Inoculating Tool.—In the course of investigations on stem rot of giant cactus[1] (*Cereus giganteus* Englm.) an inoculating tool was developed that might be useful for similar work on other succulents. This instrument, consisting of a glazier's putty gun fitted with a piece of ¼-inch copper tubing about 12 inches long (Fig. 1, A), was devised to introduce infected cactus tissue into a *C. giganteus* plant without undue injury to the plant or the operator.

[1] Lightle, Paul C., Elizabeth T. Standring, and J. G. Brown. A bacterial necrosis of the giant cactus. Phytopath. 32: 303–313. 1942.

The nozzle on the end of the putty gun was threaded with a pipe die and a coupling attached so that an automobile gasoline line compression fitting could be used on the end of the tubing. The purpose of the compression fitting was to permit easy removal of the copper tubing for cleaning.

In use, the barrel of the gun was loaded with fresh diseased cactus tissue which had been ground in a food chopper. A hole of desired depth was made in the cactus with a ¼-inch star drill, but any ¼-inch rod, brace bit, or borer could be used. The piece of copper tubing attached to the putty gun was then inserted well into the hole and the trigger of the gun
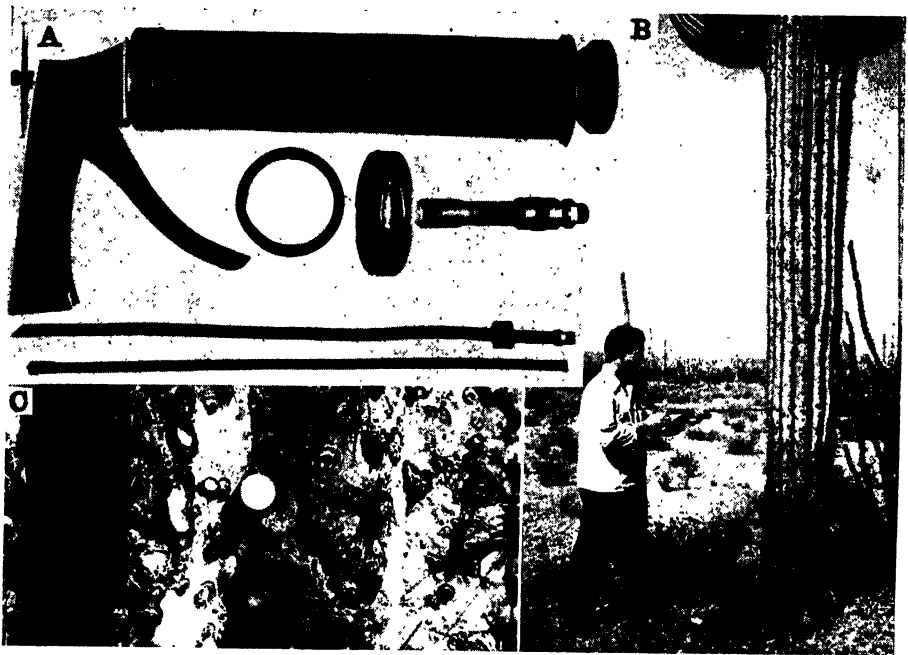
FIG. 1. A. (Top to bottom.) Putty gun, with cap detached, showing coupling on end of nozzle, leather gasket, 12-inch piece of copper tubing with compression fitting at right, and ¼-inch star drill. B. Inoculating tool in use. C. Cork plug to keep inoculum from escaping.

squeezed (Fig. 1, B). As the tubing was withdrawn from the plant the inoculum in the barrel was forced out and filled the hole. A cork was pressed into the opening to close the hole (Fig. 1, C).

The apparatus was washed out with a 1:1000 mercuric chloride solution before and after each series of inoculations. No attempt was made to completely sterilize the tool because fresh diseased tissue was used for inoculum and surface sterilization of the cactus plant with subsequent protection from contamination was considered impractical.

With this device about 20 cc. of inoculum can be quickly injected into a plant without appreciable injury to the cactus and with comparative

safety to the operator from the cactus spines.—PAUL C. LIGHTLE, Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

Infection of Seedling Peach Stems by Zoospores of Phytophthora cactorum.—In 1935 Dunegan[1] described a disease of peach seedlings caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet. Inoculations with mycelium from pure cultures of the organism demonstrated that *P. cactorum* would invade wounds made on succulent peach stems, but plants so inoculated did not wilt. Inoculation experiments in 1947 with mycelium likewise resulted in failure to produce typical wilting.

Field studies of the disease in Arkansas nurseries, particularly during the abnormally wet spring of 1945, led to the theory that water-borne zoospores are the infective agents involved in the production of the disease in the nursery rows. The fact that *Phytophthora cactorum* produces zoospores rapidly; the development of the cankers above the soil line; the occurrence of the disease only in seasons of high rainfall; and the definite localization of outbreaks of the disease to areas in the nurseries subject to flooding may be cited in support of this theory.

By using a culture of *Phytophthora cactorum* isolated from a peach seedling in Arkansas in 1945, zoospore formation was induced by the procedure described by Drechsler.[2]

To simulate natural infection conditions, soil leachate containing motile zoospores[3] was poured into water-tight cups formed by attaching small sections of plastic tubing with cellulose tape and paraffin to the seedling stems.

In the first inoculation experiment the soil leachate containing motile zoospores was used to fill the cups around 10 seedlings. As a control, sterile-soil leachate was added to the cups of an additional 10 seedlings. Typical symptoms (i.e., watersoaked streaks) appeared in the stems of all the inoculated plants at the end of 3 days, and at the end of 5 days the typical girdling cankers appeared on these plants. Seedlings wilted and finally collapsed. No pathological symptoms developed on any of the seedlings used as controls.

Seedlings also were inoculated by transferring loops of soil leachate containing zoospores to the liquid in the cups. Each loop was examined microscopically to determine that only zoospores were used in the inoculum. Typical symptoms developed on the inoculated plants within 48

[1] Dunegan, J. C. A Phytophthora disease of peach seedlings. Phytopath. 25: 800–809. 1935.

[2] Drechsler, Charles. Repetitional diplanetism in the genus Phytophthora. Jour. Agr. Res. [U.S.] 40: 557–573. 1930.

[3] Blocks 1 sq. cm. in area were cut aseptically from a 7-day-old culture of the organism in oatmeal agar, placed in sterile Petri dishes, and flooded with sterile soil-leachate. The dishes were held at room temperature 3 to 5 days until numerous zoosporangia had developed and matured in the liquid surrounding the agar blocks. When the covers were removed from the Petri dishes the zoosporangia germinated readily, and liberated swarms of motile zoospores.

hours; the stems were girdled with cankers at the end of 5 days; and the seedlings were dead at the end of 7 days. No pathological symptoms developed on the noninoculated check plants.

Finally, to complete the chain of evidence that zoospores are capable of producing the stem-canker disease of peach seedlings, the fungus was reisolated from the experimentally diseased plants and grown in pure culture, and zoospore formation was induced. These zoospores, from pure cultures obtained from the inoculated plants, were used in a third series of inoculations and produced typical cankers followed by the wilting of infected plants.

These experiments prove that zoospores of *Phytophthora cactorum* are capable of producing typical symptoms of the stem-canker disease, and the results support the theory that zoospores are the agents that induce field infections.—JOYCE E. KEPHART and JOHN C. DUNEGAN, Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

Aster Yellows in Shallot and Gladiolus.—Aster yellows is one of the most common plant virus diseases, and is known to affect a large number of plants. Only recently the first monocot was added to the list, when the susceptibility of onions was reported independently by KenKnight,[1] Larson and Walker,[2] and the writers.[3] In the present note evidence of susceptibility of two additional monocots—shallot (*Allium ascalonicum* L.) and gladiolus (*Gladiolus* sp.)—is presented.

Tims[4] has reported the occurrence of a trace to as much as 5 per cent of a disease considered likely to be aster yellows in commercial shallots in Louisiana. He describes the affected bunches as bright yellow, unmottled, and having slender leaves. Such plants fail to form bulbs, so that the disease in this host is nearly self-eliminating. A number of sample bunches supplied the writers by Dr. Tims have lived for a time in greenhouse pot culture and have exhibited the symptoms he described, but have never produced bulbs or survived to a second season. The disease is not easily confused with yellow dwarf, which is characterized by distinct yellow mottling in the shallot as in the onion.

No transmission from shallot has been attempted, but aster yellows has been transmitted from chrysanthemum (*Chrysanthemum hortorum* Bailey) to shallot, the same symptoms having been noted in naturally affected plants. The chrysanthemum source plants of the varieties Mary Lennon

[1] KenKnight, Glenn. The aster yellows disease of truck crops in Idaho: Idaho Agr. Exp. Sta. Mimeo-leaflet 79. 1943.

[2] Larson, Russell, and J. C. Walker. Aster yellows a hazard in onion seed production. *In* Wis. Agr. Exp. Sta. Bul. 463, p. 50. 1944.

[3] Brierley, Philip, and Floyd F. Smith. Some virus diseases of *Alliums*. (Abstr.) Phytopath. **34**: 990. 1944.

[4] Tims, E. C. Some shallot diseases in Louisiana. U. S. Dept. Agr., Plant Dis. Rptr. **30**: 335–338. 1946. [Processed.]

Hall and Silver Ball were supplied by Lacey McColloch from a garden in Falls Church, Va. Ten six-spotted leafhoppers (*Macrosteles divisus* (Uhl.)) were fed on these source plants from December 2 to 8, 1946, moved to China aster (*Callistephus chinensis* (L.) Nees) until December 16, then fed on 10 vigorous shallot plants from December 16 to 18. The leafhoppers became established more readily on shallot than on onion. They all fed well and survived to the end of the test. Although the shallot plants
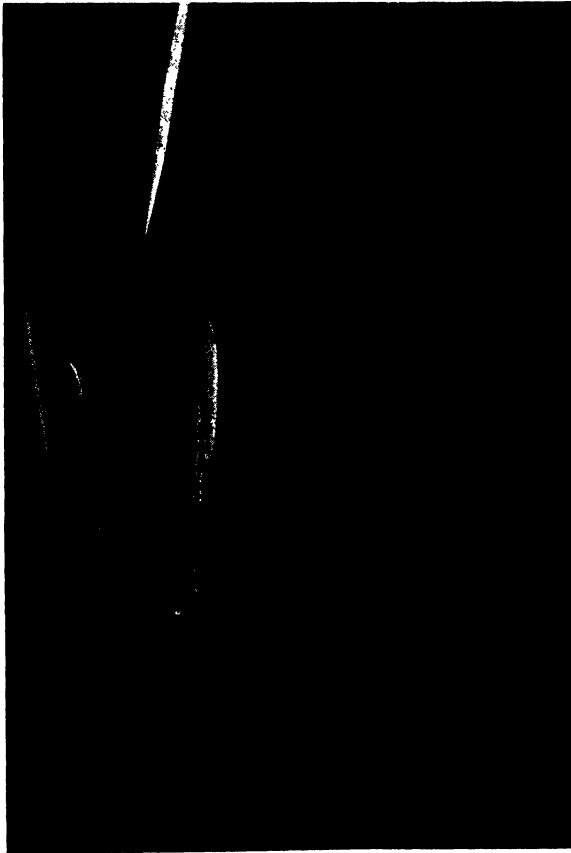
FIG. 1. Aster yellows in gladiolus (left), normal plant (right).

developed rapidly, no symptoms were detected until February 27, 1947, when one plant was noted to be somewhat more slender and upright than normal and uniformly bright yellow. None of the other plants contracted the disease. The yellow plant failed to produce a bulb and was shriveled and dead by May 9. The unaffected plants in the same pot produced normal bulbs.

Although no reisolation from the affected shallot was attempted, the close duplication of natural symptoms in this plant is considered sufficient proof that the disease was aster yellows. It is not surprising that shallot

should prove susceptible now that onions are known to be subject to the disease, as these two species are closely related. Since shallots seem to be more attractive to *Macrosteles*, aster yellows may be expected to be more troublesome on these plants than on onions.

In August, 1946, W. D. McClellan brought to our attention a single Picardy gladiolus spike that was completely virescent out of thousands of normal spikes in his plot at Beltsville, Md. All the flower parts remained green, but no proliferation was evident. The flowers eventually turned yellow and shriveled without developing the usual pink coloration. The gladioli of a seedling clone were exposed to 10 viruliferous *Macrosteles divisus* leafhoppers from chrysanthemum from December 2 to 8, 1946, when the foliage was 8 to 14 inches high. On February 14 symptoms were first detected in 1 plant. The young leaves were uniformly yellowish green, and the flower spike was spindling and green (Fig. 1). On February 24 the same symptoms appeared on a second plant. Both plants turned straw-yellow and shriveled prematurely without having shown any pink color in the flowers.

Aster yellows thus kills the tops of gladiolus rapidly after symptoms become evident. The corms developed on the two plants experimentally infected were still sound on June 20, 1947, and were beginning to produce new shoots with yellows symptoms. Aster yellows was readily transmitted to China asters from the inoculated gladiolus by means of *Macrosteles divisus*. Forty nonviruliferous nymphs were fed on an infected gladiolus flower for 2 days and then transferred to five China asters on March 8, 1947. By April 11 all the plants had developed typical symptoms of aster yellows.

The susceptibility of gladiolus to this virus is thus fully confirmed. The rarity of the disease in this crop is surprising inasmuch as the vector, *Macrosteles divisus*, is frequently found feeding on gladiolus in the field. —FLOYD F. SMITH, Bureau of Entomology and Plant Quarantine, and PHILIP BRIERLEY, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture.

BOOK REVIEWS

CARTWRIGHT, K. ST. G., and W. P. K. FINDLAY. *Decay of Timber and its Prevention.* Department of Scientific and Industrial Research publication, 294 pp., illus. 1946. Published by H. M. Stationery Office, London. 12s. 6d.

This is the first modern book in English dealing exclusively and comprehensively with timber and wood decay. The authors are eminently qualified to write in this field having worked together in it for many years at the Forest Products Research Laboratory, Princes Risborough, Aylesbury, Buck, England. The subject of decay and other fungus defects of wood, and methods of controlling them, is now developed to a point where anything like a full treatment of it cannot be attempted in a single book of ordinary size. Faced with this situation, Cartwright and Findlay have shown commendable discrimination in their choice of phases to be considered and in the selection of literature that they have drawn on. Those wanting a better understanding of the factors contributing to decay and of the causal fungi and their effect on wood, and those concerned with the prevention of decay will find this a valuable text book and reference source.

The contents are taken up under 14 main headings: introduction (economic factors and historical); causes of decay; technique (isolation of fungi, cultural and staining method, etc.); physiology of wood-destroying fungi; effects of fungus decay on wood; principal decays of standing trees in Great Britain (two chapters, coniferous, and broad-leaved trees); rots of felled timber and of timber in service in the open; rots of timber in buildings and structures; prevention of decay in felled and converted timber during storage and shipment; decay of timber in buildings, mines, vehicles, aircraft, ships and boats, sleepers, poles, fencing and in horticultural use; deterioration of composite wood and manufactured wood products; natural durability of timber; preservation of wood by chemicals, and staining and discoloration of timber. There is also an appendix in which is given the British standard method of test for the toxicity of wood preservatives. This method in its general aspects is used in many laboratories outside Great Britain. Sub-headings are freely used, making for rapid location of individual topics and clarity of presentation.

In the chapters dealing with the rots of standing trees, felled timber, and buildings, emphasis is placed on the causal fungi and their characteristics. Descriptions of the cultural and physiological characteristics of the more important fungi should be of special interest to many readers as information on these points has been rather difficult to find. For the same reason, many will find the chapter dealing with the physiology of wood-destroying fungi very helpful. Remedial and preventive measures are considered to some extent with reference to the individual fungi, in the felled timber and buildings, whereas general measures are considered in subsequent sections.

Although British conditions naturally receive considerable attention in places, North American readers will not find this excessive for the most part. The chapters of most marked regional character in this respect are those on the decay of standing trees. A few things might be added to the book in any subsequent revision that would further its value to American users. For example, it would seem desirable to list more of the commercial timbers falling in the durable to moderately durable groups. Brush treatment of stored logs probably would be less favored than a spray treatment in the United States and Canada, except where an end coating to control checking is needed. It is also probable that one of the anti-stain chemicals used on lumber would find more favor for log treatment than creosote. In addition to sodium pentachlorophenate for the preservation of wet pulp during storage, mention might be made of ethyl mercuric phosphate. This compound has given a good account of itself in Swedish investigations and is used commercially in this country. Other new organic fungicides are coming into use for the same purpose. "Wood Preservation," by Hunt and Garratt should be cited as it is an important modern text and reference book in English on this subject.—THEODORE C. SCHEFFER, Forest Products Laboratory, Madison, Wisconsin.

WHITEHEAD, S. TATUM, THOMAS P. MCINTOSH, and WILLIAM W. FINDLAY. *The potato in health and disease.* 400 + XV pages, 31 figs. Oliver and Boyd, London. 1945. 25s.

The first half of this book is devoted to the potato plant and its culture, and the second to potato diseases. In the first section the origin and taxonomy of the cultivated potato is discussed, and considerable space is devoted to the morphological characters by which varieties are distinguished. An appendix gives the descriptions of some 50 British varieties; their distinguishing characteristics; cultural, cooking, and keeping qualities; and their reactions to diseases, especially those caused by viruses.

Cultural methods used in the British Isles are described in detail, with references to scientific literature on various phases of potato growing. Seed certification standards and methods of particular interest to seed growers are discussed.

The section on pathology has a key to parasitic diseases and abnormalities caused by insects and non-parasitic agencies. Of the 19 parasitic diseases that are discussed in detail some, such as that due to *Armillaria mellea*, would be considered of little importance in the United States, while ring-rot (*Corynebacterium sepedonicum*) is not mentioned. The parasitic diseases are classified on the basis of pathogen taxonomy, and the characteristics and life histories of the pathogens discussed in considerable detail. A useful feature of the description of symptoms is the attention given to characteristics by which each disease may be distinguished from others with similar symptoms.

The discussion of virus diseases of the potato occupies nearly 100 pages, and covers all phases of the subject, including descriptions of the principal virus diseases, the reactions of varieties to different viruses -and virus complexes, means of transmission, descriptions of aphid vectors, and practical problems encountered in control.

Judging from the simple way in which basic biological concepts, such as cell division and inheritance, are described, this book is meant to appeal to people with little background in biology. This does not mean that it is superficial or unauthoritative. It will be useful to anyone wishing a scientific reference on potato growing and disease control. Growers, and especially seed certification officials, should find it very valuable, and it would be useful to any investigator working on potato problems.—C. J. EIDE, Minnesota Agricultural Experiment Station.

CHESTER, K. STARR. *The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat.* 269 pp., 11 figures, 19 tables. Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York City. 1946. $5.00.

This book is an extensive review of the world literature on leaf rust of wheat and should be welcomed by students and research workers as a valuable reference to the subject, particularly at the present time when the disease is assuming increasing economic importance in the United States. Dr. Chester is to be commended for his efforts in translating the pertinent Russian literature on the subject which, due to difficulties in translating, is made available to many for the first time. The book is more than a summary of the literature. Many of the data have been analyzed and suggestions made that will be helpful in continuance of research on the subject.

There are fifteen chapters in the book with titles serving as guides to the contents. In chapter 1 is a history of the disease up to the present time. In chapters 2 and 3 are discussed the origin, distribution, economic importance, and the effect of the rust on the host plant and its yields. Here the author has presented rather conclusive data to show that in the world as a whole leaf rust is even more destructive than stem rust. Those, in North America at least, who have witnessed several of the devastating stem rust epidemics will undoubtedly be skeptical on this point. Chapter 4 deals primarily with suscepts. Information is presented on the host range of the pathogen and on the reactions of wheat varieties together with a review of results of investigations on the alternate hosts. Noteworthy is the fact that *Isopyrum fumarioides*, although present in North America as well as in other parts of the world, functions as an alternate host only in eastern Siberia. Chapters 5 and 6 deal with the symptoms of the disease on the economic and alternate hosts, and review the cytology and sexuality of the pathogen as determined primarily -by the work of Allen. Plant breeders as well as pathologists will be particularly interested in Chapters 7 and 8, which deal with physiologic specialization. The author suggests eliminating three somewhat unstable differential varieties and on the basis of similarities classifies the known 129 races in 44 race groups. Both suggestions have considerable merit and if the data obtained on this basis appear adequate for the needs in our present day breeding programs, the proposed system will simplify to some extent the work of both pathologists and breeders. In Chapters 9 and 10 are considered the factors affecting the survival and development of the rust. Environmental factors such as moisture, temperature, light, physical conditions, and chemical constitution of the soil are discussed at some length. There is included also a discussion on studying, analyzing, and expressing combined environmental factors together with suggestions for correlating data of value for forecasting of epidemics at least in limited areas. The practical application will be open to question in the minds of many. Chapter 11 deals with rust dissemination, annual cycles, and epiphytotics. Dissemination of rust spores by various agents, evidence of long distance dissemination in different parts of the world, and annual cycles are adequately discussed. The last few chapters of the book are devoted to rust control by natural, regulatory, and cultural means, by the use of fungicides, and by the development of resistant varieties. The advantages and limitations of the different methods are adequately discussed. There is also a good discussion of the literature on results of breeding for leaf rust resistance. In fact, throughout the book the reader is impressed with the exhaustive search and digest of the literature that Dr. Chester has made to present a complete summary of the information on the subject.—H. A. RODEN-HISER, Bureau of Plant Industry Station, Beltsville, Maryland.

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FUSARIUM DISEASES OF BROAD BEAN. III. ROOT–ROT AND WILT OF BROAD BEANS CAUSED BY TWO NEW FORMS OF FUSARIUM[1]

T. F. Y u a n d C. T. F a n g [2]

INTRODUCTION

Foot-rot, root-rot, and wilt are very destructive diseases in the broad-bean growing regions of Yunnan, China, especially where proper rotation has not been practiced. They have been found in all regions where beans are grown extensively and in certain sections have caused annual losses as high as 40 per cent of the crop.

The causes of these diseases were found to be species of *Fusarium*. One of them is *Fusarium avenaceum* var. *fabae* which causes a wilt disease. The results of an investigation of the morphology and physiology of this fungus together with the disease induced by it have been reported in preceding papers (8, 9). In the present paper, two additional species of *Fusarium* parasitizing broad bean are described.

THE ROOT-ROT FUNGUS

Cultural Characters of Fusarium solani f. fabae n.f.

Hard potato agar. Cultures 1 month old are characterized by a loose, coarse, wooly, granular, white to cartridge buff mycelium (4, 5). Pionnotes, produced abundantly in large heaps, are putty buff and cream buff. Older cultures have thick and more or less compact mycelial growth that is laelia pink, Burgundy purple, and Rigi blue. Sclerotia are small, Rigi blue to slate color, and usually imbedded in the mycelia. Numerous India buff sporodochia are produced.

Hard oat agar. Cultures 1 month old are characterized by a medium coarse to fine wooly mycelium that is white to buff. Sporodochia and pionnotes are produced in large groups over the slant. Sclerotia are produced abundantly in masses and are spruce green to Rigi blue.

Broad-bean seed-decoction agar. Mycelium is scant, white, and cottony on 12-day-old cultures; scant, white, and wooly on one-month-old cultures. Small putty-buff sporodochia are generally formed on the basal portion of the slant. Sclerotia are not seen.

Potato-tuber plug. Cultures 1 month old have a loose, coarse, wooly, granular mycelium that is white, light Martius yellow, and Rigi blue. Large masses of sporodochia that run together, forming pionnotes, are usually produced. They are usually cinnamon to powdered yellow. The abundant sclerotia are blue spruce green to Rigi blue.

Melilotus stem. One-month-old growth is characterized by a loose, coarse, wooly, granular, white to buff mycelium. Cinnamon to powdered yellow pionnotes and sporodochia are present over the stem in small and large heaps. Small, Rigi blue sclerotia may be present.

Green broad bean pod. Cultures 2 months old are characterized by a medium thick, matted, cartridge buff and ivory buff mycelium. Large, spherical cinnamon sporodochia are produced. Sclerotia are small and Rigi blue.

Broad bean stem. Cultures 1 month old have scant, white to cartridge buff aerial mycelium. Small, pale pinkish buff sporodochia are produced.

Rice. Cultures 12 days old have a moderately compact, matted mycelium that is white, cartridge buff and rosewood red, while the rice is changed to coral pink. Small, Rigi blue sclerotia are developed. Cultures 1 month old are characterized by scant, white, aerial mycelium. The rice is rosewood red to prune purple and Rigi blue to slate color. Sporodochia are putty buff to sheepskin moth yellow, single or aggregated, and circular or irregular in outline.

Potato agar plate, 5 per cent dextrose. Cultures 1 month old have a white to cartridge buff aerial mycelium, and that in the substratum is cinnamon buff and rosewood red. The cultures are zonate. Sporodochia may be present in the center of the plate. Microconidia are produced in great abundance.

Measurements of Conidia from Sporodochia Produced on Different Media

Hard oatmeal agar; cultures 44 days old:

    3-septate, 57 per cent, 38.8 × 5.2 (31.8–44.1 × 4.8–5.4) μ.
    4-septate, 21 per cent, 40.9 × 5.2 (35.3–49.4 × 4.9–5.3) μ.
    5-septate, 22 per cent, 42.7 × 5.2 (38.8–47.6 × 3.9–5.3) μ.

Cornmeal agar; cultures 44 days old:

    3-septate, 78 per cent, 34.4 × 5.4 (23.6–40.0 × 3.6–5.5) μ.
    4-septate, 13 per cent, 40.3 × 5.5 (32.7–50.9 × 5.3–5.8) μ.
    5-septate, 9 per cent, 43.3 × 5.6 (38.2–47.3 × 5.5–6.2) μ.

Diseased broad bean plants; plants killed two weeks after inoculation:

    0-septate, 1 per cent, 19.4 × 3.0 μ.
    1-septate, 2 per cent, 21.7 × 3.4 (21.2–22.9 × 3.2–3.7) μ.
    2-septate, 2 per cent, 32.6 × 4.8 (31.8–33.5 × 4.4–5.3) μ.
    3-septate, 82 per cent, 32.9 × 4.7 (26.5–42.4 × 3.5–5.3) μ.
    4-septate, 7 per cent, 36.7 × 5.0 (31.9– 4.2 × 3.5–5.3) μ.
    5-septate, 6 per cent, 38.6 × 4.9 (35.3–42.4 × 3.9–5.3) μ.

Hard potato agar, 2 per cent dextrose; cultures 44 days old:

    1-septate, 2 per cent, 20.0 × 3.3 μ.
    2-septate, 4 per cent, 21.8 × 3.7 (20.0–23.6 × 3.6–3.8) μ.
    3-septate, 78 per cent, 32.4 × 5.5 (25.5–40.0 × 4.0–5.8) μ.
    4-septate, 12 per cent, 35.8 × 5.5 (32.7–38.2 × 5.5–5.6) μ.
    5-septate, 4 per cent, 38.2 × 5.5 (41.5–48.5 × 5.1–5.6) μ.

Broad bean seed-decoction agar; cultures 138 days old:

    3-septate, 13 per cent, 30.6 × 5.5 (26.5–34.4 × 5.3–6.2) μ.
    4-septate, 22 per cent, 35.6 × 5.5 (31.8–42.4 × 5.3–6.5) μ.
    5-septate, 62 per cent, 40.5 × 5.6 (32.6–47.6 × 5.1–7.1) μ.
    6-septate, 3 per cent, 44.6 × 5.5 (41.5–48.5 × 5.1–5.6) μ.

Potato-tuber plug; cultures 30 days old:

    3-septate, 82 per cent, 33.7 × 5.3 (26.5–45.9 × 4.1–5.9) μ.
    4-septate, 12 per cent, 36.0 × 5.2 (32.6–40.6 × 4.8–5.3) μ.
    5-septate, 6 per cent, 38.8 × 5.3 (35.3–42.4 × 5.1–5.4) μ.

Melilotus stem; cultures 44 days old:

    3-septate, 37 per cent, 34.9 × 5.3 (23.6–41.8 × 4.2–5.6) μ.
    4-septate, 44 per cent, 38.1 × 5.4 (32.7–45.5 × 5.1–5.5) μ.
    5-septate, 19 per cent, 40.2 × 5.4 (36.4–43.6 × 4.7–5.5) μ.

Green broad bean pod; cultures 138 days old:

    3-septate, 36 per cent, 33.5 × 5.1 (27.4–37.1 × 3.9–5.3) μ.
    4-septate, 30 per cent, 36.5 × 5.2 (32.6–42.4 × 4.8–5.6) μ.
    5-septate, 33 per cent, 37.8 × 5.6 (32.6–42.4 × 4.6–5.3) μ.
    6-septate, 1 per cent, 38.8 × 5.3 μ.

Green pea pod; cultures 138 days old:

    3-septate, 55 per cent, 32.5 × 5.1 (28.2–40.6 × 3.9–5.6) μ.
    4-septate, 23 per cent, 33.7 × 5.2 (27.3–37.1 × 3.9–5.6) μ.
    5-septate, 22 per cent, 38.8 × 5.3 (33.6–40.6 × 3.9–5.6) μ.

Robinia root; cultures 32 days old:

    3-septate, 44 per cent, 34.1 × 5.3 (26.5–38.1 × 4.1–6.0) μ.
    4-septate, 30 per cent, 37.0 × 5.3 (31.8–42.4 × 4.6–5.6) μ.
    5-septate, 26 per cent, 38.8 × 5.3 (31.8–42.4 × 4.9–6.2) μ.

Steamed rice; cultures 44 days old:

    3-septate, 44 per cent, 39.0 × 5.2 (26.5–46.8 × 4.1–5.2) μ.
    4-septate, 19 per cent, 41.6 × 5.2 (31.8–46.8 × 4.1–5.5) μ.
    5-septate, 37 per cent, 43.2 × 5.3 (35.5–52.8 × 4.1–5.4) μ.

The averages for conidia on different media of various ages are as follows:

    0-septate, 19.4 × 3.0 μ.
    1-septate, 20.8 × 3.3 (20.0–21.7 × 3.3–3.4) μ.
    2-septate, 27.2 × 4.2 (21.8–32.6 × 3.7–4.8) μ.
    3-septate, 34.8 × 5.2 (30.6–39.0 × 4.8–5.5) μ.
    4-septate, 38.3 × 5.2 (35.5–41.6 × 4.9–5.5) μ.
    5-septate, 40.2 × 5.3 (38.2–43.3 × 4.9–5.6) μ.
    6-septate, 44.9 × 5.3 (38.8–53.4 × 5.0–5.5) μ.

Morphology of the Fungus

Microconidia borne on irregularly branched conidiophores, ovoid, oblong, or short rod-shaped, 6.6 × 2.1 μ, rarely 1-septate, 12.8 × 2.6 μ; macroconidia scattered in mycelium or in false heads, in sporodochia or pionnotes, spindle-shaped or slightly curved, with rounded to slightly constricted apex, slightly or not at all pedicellate, 0- to 6-septate, typically 3-septate, 34.8 × 5.2 μ; conidial mass gold yellow, putty buff, cinnamon or cream colored; sclerotia small, Rigi blue or slate color; chlamydospores terminal or intercalary, mostly 1-celled, spherical or oblong, 10.6 × 10.0 μ, 2-celled, 24.2 × 15.1 μ, sometimes in short chains, smooth or sometimes rugose (Fig. 1, A, B, and C).

This fungus, isolated from diseased broad bean root and proven to be parasitic on the host, is practically identical with *Fusarium solani* (Mart.) App. et Wr. (7), especially in spore measurements and cultural character-

Potato-tuber plug. Cultures 22 days old have a medium dense, leathery, dirty white mycelium, sometimes with spots of sheepskin moth yellow, slate color, dark chessylite and blue-green in places. Sclerotial stromata are not present.
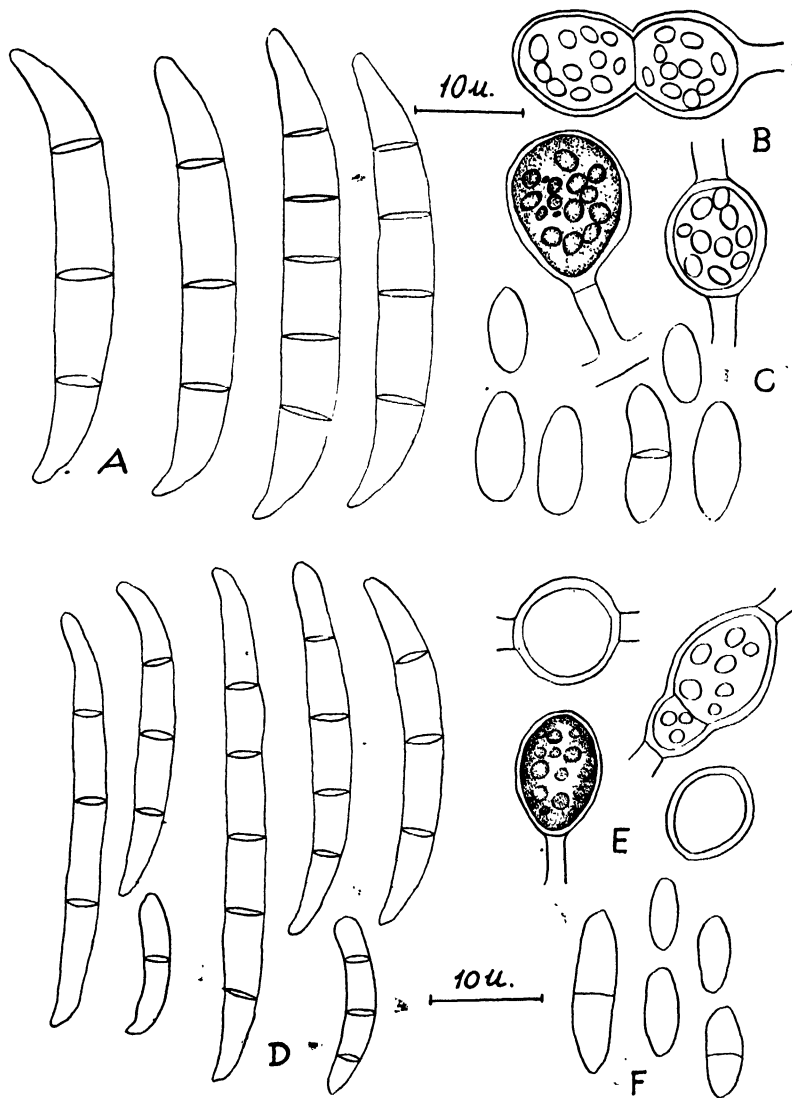
Fig. 1. *Fusarium solani* f. *fabae:* A. Macroconidia; B. Chlamydospores; C. Microconidia. *Fusarium oxysporum* f. *fabae:* D. Macroconidia; E. Chlamydospores; F. Microconidia.

Melilotus stem. Cultures 1 month old have a medium scant, white, tawny, and Arnoblue mycelium with occasional tufts that are white and wooly. Sclerotial stromata may be present.

Green broad bean pod. Cultures 5 months old have a medium compact

mycelium that is dirty white and Rigi blue. The mycelium may be slimy or self-digested.

Green pea pod. The cultural characters of the fungus on pea pod are the same as on broad bean pod except that in a few instances small brown sporodochia may be produced. Sclerotial stromata are not present.

Rice. Cultures 12 days old have a medium dense, dirty white mycelium. Older cultures, from 1 to 2 months old, are characterized by a leathery mycelium that is La Valliere to dahlia purple, Crown Prince gray, dusky blue, and slate color. There are also spots of cream-buff, yellow ocher, coral pink, and Isabella color.

This fungus produces relatively abundant microconidia and sometimes sclerotial stromata on these cultural media. However, macroconidia are rarely produced, and sporodochia or pionnotes are often absent. The mycelial growth is at first dirty white, compact, and dense. In most of the tube cultures the presence of a greenish blue narrow lip on the lower edge of the slant surface is very characteristic.

Measurements of Conidia on Different Media

Diseased broad bean plants; conidia from aerial mycelium:

    1-septate, 3 per cent, 23.2×3.5 $(22.0–23.8 \times 3.4–3.9)$ μ.
    2-septate, 3 per cent, 26.1×3.6 $(26.7–27.3 \times 3.5–3.9)$ μ.
    3-septate, 91 per cent, 35.8×3.6 $(25.6–44.1 \times 3.2–5.3)$ μ.
    4-septate, 2 per cent, 37.9×4.1 $(35.3–40.6 \times 3.7–4.4)$ μ.
    5-septate, 1 per cent, 47.6×4.9 μ.

Diseased broad bean plants in moist chamber; cultures 30 days old; conidia from aerial mycelium:

    1-septate, 29 per cent, 21.0×3.5 $(14.1–26.4 \times 3.2–4.4)$ μ.
    2-septate, 11 per cent, 26.8×3.6 $(24.6–29.9 \times 3.3–3.9)$ μ.
    3-septate, 54 per cent, 32.8×4.0 $(22.9–51.0 \times 3.5–5.3)$ μ.
    4-septate, 6 per cent, 39.9×4.6 $(35.2–46.2 \times 3.5–5.3)$ μ.

Pea pod; cultures 74 days old; conidia from sporodochia:

    3-septate, 90 per cent, 29.2×3.8 $(21.8–36.4 \times 3.5–4.9)$ μ.
    4-septate, 8 per cent, 35.1×3.7 $(23.6–34.5 \times 3.5–3.8)$ μ.
    5-septate, 2 per cent, 37.3×4.0 $(36.4–38.2 \times 3.8–4.2)$ μ.

Melilotus stem; cultures 48 days old; conidia from aerial mycelium:

    1-septate, 4 per cent, 23.2×3.3 $(15.4–26.1 \times 3.2–4.4)$ μ.
    2-septate, 4 per cent, 25.5×3.3 $(24.6–27.8 \times 3.2–4.3)$ μ.
    3-septate, 88 per cent, 35.1×4.0 $(29.9–47.5 \times 3.5–4.9)$ μ.
    4-septate, 4 per cent, 43.0×4.6 $(39.8–48.1 \times 3.5–4.9)$ μ.

Robinia root; cultures 74 days old; conidia from aerial mycelium:

    1-septate, 1 per cent, 15.0×2.1 μ.
    2-septate, 1 per cent, 15.9×3.0 μ.
    3-septate, 91 per cent, 24.7×3.3 $(17.6–37.1 \times 2.6–3.9)$ μ.
    4-septate, 4 per cent, 33.5×3.4 $(26.5–37.1 \times 3.2–3.5)$ μ.
    5-septate, 3 per cent, 33.7×3.5 $(31.8–38.8 \times 3.2–3.7)$ μ.

The averages of these measurements of conidia on different media of various ages are as follows:

    0-septate, 8.9×2.9 μ.
    1-septate, 17.8×3.0 $(9.9–23.2 \times 2.1–3.5)$ μ.

2-septate, 25.7 × 3.5 (15.9–26.8 × 3.0–3.6) μ.
3-septate, 31.9 × 3.7 (24.7–35.8 × 3.3–4.0) μ.
4-septate, 38.0 × 4.1 (33.5–43.0 × 3.4–4.6) μ.
5-septate, 40.6 × 4.1 (33.7–47.6 × 3.5–4.9) μ.

Morphology of the Fungus

Mycelium vigorous, dirty white at first, becoming tawny to variously tinted, pigment of substratum highly variable; microconidia formed free in aerial mycelium, relatively abundant, ovoid, oblong or irregularly ellipsoid, mostly 0-septate, 7.1 × 3.3 (5.2–10.4 × 2.1–3.5) μ; macroconidia rarely produced, mostly 3-septate, nearly uniform in diameter or slightly broader toward the upper end, slightly curved at the upper end, usually with rounded apex and with base nearly straight-conical or appendicular, seldom subpedicellate, 31.9 × 4.1 μ; sporodochia and pionnotes rarely produced; sclerotial stromata present, in certain cultural media, Highland green and spruce green to marble green; chlamydospores single, in pairs, or in short chains, terminal or intercalary, spherical to subspherical, thick-walled, dark brown, smooth, 7.3 × 6.9 (6.6–9.1 × 5.8–7.4) μ (Fig. 1, D, E, and F).

The foregoing description of the fungus causing wilt of broad bean shows that it resembles *Fusarium oxysporum* emended by Snyder and Hansen (6) and it especially resembles *F. oxysporum* f. *pisi* (3, 7) in both cultural and morphologic characteristics. It differs, however, from the pea fungus in pathogenicity. Cross inoculation experiments were made with this fungus and two cultures of *F. oxysporum* f. *pisi*. One of these pea fungus cultures was received through the kindness of Dr. J. C. Walker of United States and the other was isolated by the writers from peas in Yunnan. The results of these experiments indicate the pea fungi occasionally cause infection on broad bean. On the other hand, it has been impossible so far to cause infection on peas with the broad bean fungus. Following the system of classification of *F. oxysporum* proposed by Snyder and Hansen (6), the present fungus will be named *F. oxysporum* f. **fabae** as a new form of this species.

Pathogenicity

The most characteristic symptom of the disease caused by this fungus on the aerial portion of broad beans is the yellowing of the leaves. Affected leaves become yellow and more or less rigid. The roots and basal portion of the stems of a diseased plant are not conspicuously discolored. When the diseased roots are cut open, the vascular tissues have the characteristic reddish brown discoloration.

Inoculations of beans, cowpeas, peas, oats, wheat, and corn with this fungus were negative.

RÉSUMÉ

Two new forms of *Fusarium* that cause diseases in *Vicia faba* L. are reported in this paper. One of them, causing the root-rot of broad bean,

is named *F. solani* f. *fabae* and the other, causing wilt of the same host, is named *F. oxysporum* f. *fabae*. Their cultural characteristics, morphology, and pathogenicity are described.

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THE DEVELOPMENT OF GIBBERELLA ZEAE HEADBLIGHT OF WHEAT

AXEL L. ANDERSEN

INTRODUCTION

Although wheat headblight or scab caused by *Gibberella zeae* (Schw.) Petch has been investigated frequently during the past 60 years, some aspects of the disease still need clarification. The literature has been reviewed adequately by Atanasoff (2), MacInnes and Fogelman (9) and Eide (6).

This paper includes studies on the effects of temperature on growth, sporulation, and spore germination of the pathogen; the effects of the inoculum dosage, stage of host maturity, and air temperature and air moisture on disease development.

MATERIALS AND METHODS

Laboratory Studies

A single monoconidial isolate of *Gibberella zeae* was used throughout the investigations. This culture was selected from several obtained from Dr. J. J. Christensen of the University of Minnesota, because of its pathogenicity on seedling wheat.

The conidial stage of *Gibberella zeae* (*Fusarium graminearum* Schwabe) was produced on Coons' agar initially adjusted to pH 3.2 using normal HCl. This medium was used in the temperature studies on growth and sporulation and also for producing the conidia used for plant inoculation.

The effect of temperature on growth, sporulation, and germination was studied in incubators maintained within $\pm 0.5°$ C. of the desired temperature. Growth rates were determined on agar in Petri plates which had been seeded with a 5-mm. agar plug in the center of the plate. Diameter of the mycelia was recorded after 5 days. Methods similar to those presented by Henry and Andersen (7) were used in the sporulation studies.

Spore germination was studied by placing 2 to 4 drops of a dilute conidial suspension of *Gibberella zeae* on a clean glass slide. The slide was placed in a Petri plate during the incubation period to prevent the water from evaporating. At least 200 germinated and non-germinated conidia were counted on each slide and the percentage germination determined on the basis of these counts.

Greenhouse Studies

Newthatch spring wheat was the principal variety used in the greenhouse experiments. The wheat was sown in soil in one-gallon glazed pots and thinned to 10 plants per pot shortly after emergence. It was grown at relatively cool temperatures (12° to 15° C.) for the first two months,

and thereafter in a warm greenhouse (20° to 25° C.) with supplementary lighting. Uniform heads were obtained nearly identical in appearance to those produced on wheat grown in the field.

Plants were inoculated at five stages of development as follows: (1) the boot stage, in which the head was still enclosed in the leaf sheath; (2) the before-flowering stage, in which the heads were out of the boot but none of the flowers had extruded anthers; (3) the flowering stage, in which the flowers were in the process of anthesis or had already extruded anthers; (4) the after-flowering stage, in which small immature seeds were present and (5) the past-flowering stage in which the kernels were nearly filled out and in the milk to soft dough stage.

The technique used for plant inoculation was the same as that reported by Andersen *et al.* (1). This consisted in the application of a suspension of conidia by means of a hand-made atomizer. Unless mentioned otherwise, the standard application of inoculum in all experiments was approximately two million conidia per pot. Conidial germination was checked prior to the use of the inoculum to insure the application of viable conidia.

In all the studies on pathogenesis, the plants were exposed to continued wetness for different periods in the humidity tents before they were removed to the greenhouse bench to complete their incubation period. Greenhouses were maintained at approximately 25° C.

RELATION OF TEMPERATURE TO GROWTH, SPORULATION, AND SPORE GERMINATION IN VITRO

. The rate of growth of *Gibberella zeae* on Coons' agar was determined after five days at different temperatures. There was a very rapid increase in the rate of growth from 8° to 28° C. with an especially significant increase between 20° and 24° C. The growth rate at 32° C. was comparable to that obtained at 12° C. and no growth resulted at 36° C. Slow growth occurred at 4° C., so the minimum temperature is probably slightly below 4° C.

Studies on the effect of temperature on spore production were conducted at the same temperatures as in the growth rate studies. The most rapid spore production occurred initially at 28° and 32° C., but on longer incubation (after 7 and 12 days) considerably more conidia were produced at 32° C. (Fig. 1, A). The strain used in these studies produced very few conidia at temperatures below 20° C. and none at 36° C. Thus the best temperature for sporulation in culture was not the same as that for growth.

Microscopic observations made while making spore counts indicated that spores from cultures incubated at different temperatures differed morphologically. In order to determine whether the incubation temperatures exerted an influence on the length of the conidia, measurements and records of 20 to 25 conidia from one culture incubated at each of the temperatures favoring good sporulation (20° to 32° C.) were taken at random. At 20° and 24° C., 60 to 70 per cent of the conidia were 5-septate and the remain-
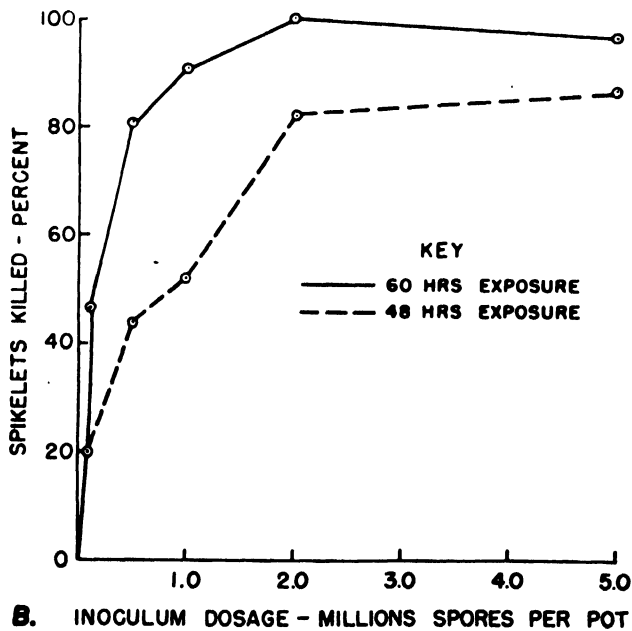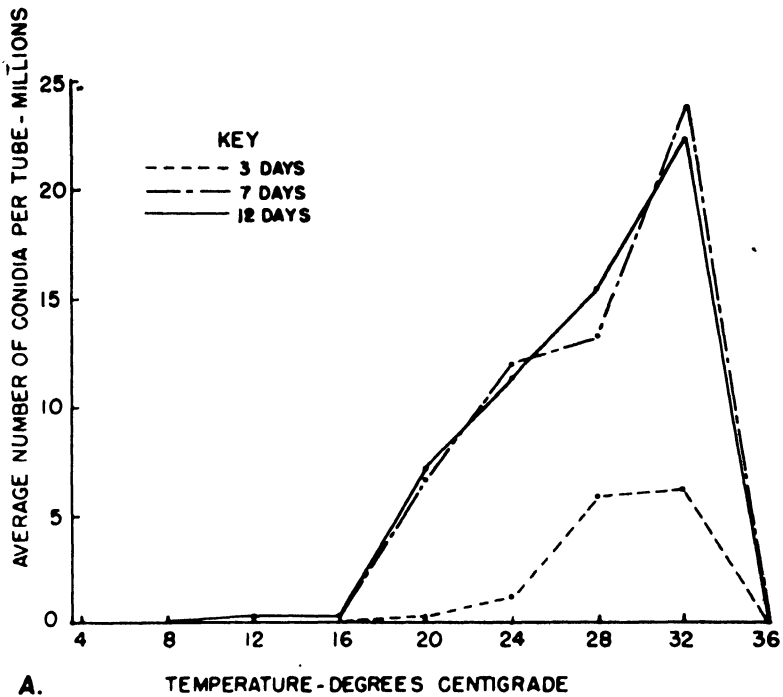
Fig. 1.   A. Effect of temperature on sporulation by *Gibberella zeae* on Coons' agar.
B. The effect of inoculum dosage on the development of Gibberella headblight on wheat
inoculated in the flowering stage.

TABLE 1.—*Effect of temperature on the germination of Gibberella zeae conidia*

Temperature, degrees C.	Time in hours			
	6	24	48	72
	Percentage germination[a]			
4	0.0	0.0	88.9	93.7
8	0.0	17.4	95.6	....
12	0.0	85.9	96.8	....
16	50.3	88.5	....	....
20	85.2	....	....	....
24	89.7	....	....	....
28	93.5	... ...	... .	....
32	91.0	....	... .	... ...
37	0.0	0.0	0.0	0.0

[a] Based upon counts of 200–400 conidia for each sample.

der 3- and 4-septate. At 28° C. only 27 per cent were 5-septate while over 40 per cent were 3-septate and the remainder 4-septate. At 32° C., over 50 per cent were 2-septate, more than 30 per cent were 3-septate, and a few were 1- and 4-septate. Conidia with a like number of septations were approximately equal in length at all temperatures. For example, the 3-septate conidia measured 37.5–41.2 μ; the 4-septate 41.0–44.1 μ; and the 5-septate 47.9–49.5 μ. Thus temperature influenced the length of the conidia produced on artificial media through a reduction in the number of septa in each conidium.

The conidia of *Gibberella zeae* germinate very rapidly. Many were observed to have germinated in 3 hours at 28° or 32° C. In 6 hours, nearly all the conidia exposed at 20°, 24°, 28°, and 32° C. had germinated (Table 1). At lower temperatures the rate of germination was considerably slower, although the percentage germination at the lower temperatures after 48 and 72 hours of incubation was as high as that recorded at 28° C. in 6 hours.

Records on the percentage germination of the conidia (Table 1) supplemented by measurements of the germ tubes (Table 2) gave more reliable

TABLE 2.—*Effect of temperature on the length of the germ tubes of Gibberella zeae*

Temperature, degrees C.	Time in hours			
	6	24	48	72
	Average length of germ tubes in microns[a]			
4	0.0	. g	38.9	137.8
8	0.0	g	146.3	....
12	0.0	51.6	....	....
16	10.6	131.8	....	....
20	31.6	....	... .	....
24	65.2	....	....	....
28	111.1	....	....	....
32	63.2	....	....	....

‡ g = short knobby germ tubes just beginning to protrude from the conidia.

information on the effect of temperature on germination as well as on growth. Measurements on germ tube length made at the end of 6 hours showed that the most rapid elongation of the germ tubes occurred at 28° C. The germ tube measurements from conidia germinated at the different temperatures coincide closely with the data on mycelial growth.

EFFECT OF INOCULUM DOSAGE ON DISEASE SEVERITY

The studies on the effect of inoculum dosage on disease severity were made to determine the approximate number of spores to apply to the plants in the remainder of the experiments on disease development. Initial experiments indicated considerable variation in the amount of infection established and in the number of heads and spikelets killed when various quantities of conidia were applied to plants. To standardize the procedures, several experiments were conducted on the effects of inoculum dosage on disease development. The results of two of these experiments are presented in figure 1, B. The amount of disease development was not the same in the two experiments, probably because the plants treated in the first experiment were exposed for 48 hours at 25° C. in the humidity tent, whereas the other series received an additional exposure of 12 hours at the same temperature. Since the application of two million conidia per pot resulted in a high percentage of infection in 48 hours at 25° C. and in 100 per cent of the heads and spikelets becoming infected and killed with 60 hours' exposure, this conidial dosage was selected as a standard for use in the remaining experiments.

RELATION OF HEAD MATURITY TO DISEASE DEVELOPMENT

The studies on the relation of inoculum dosage to disease development indicate that there is a difference in susceptibility of wheat heads to *Gibberella zeae* infection during the various stages of head maturity. Several experiments were conducted to determine the relative susceptibility of the heads and spikelets to infection from the boot to the past-flowering stage of development.

In order to determine the susceptibility in the various stages of development, several wheat heads were tagged and records were obtained on the exact stage of development of each of the spikelets on each head prior to inoculation with *Gibberella zeae*. After inoculation the plants were subjected to continued wetness for 48 hours at 25° C. Records on infection were taken 3 days after inoculation and on killed spikelets 5 days later (Table 3). In another experiment, heads in the before-flowering and flowering stages of development were tagged and observed before and after inoculation. The results were the same as those obtained in the previous experiment. No infection was observed on spikelets which had not blossomed prior to the time when the plants were removed from the humidity tent, except in the case of two spikelets which were infected at the base. The infection of one of these appeared to have resulted from contact with

an infected extruded anther from the spikelet just below it. The anther in this case was lodged between the spikelet and the rachis. On the heads showing partial anthesis, only those spikelets which had blossomed were infected. In this respect, the results appear to support the theories of Pugh, Johann, and Dickson (13) who concluded that the progress of infection of the spikelets was dependent upon anthesis. Apparently wheat heads are very resistant to infection prior to flowering.

Atanasoff (2) and Pugh, Johann, and Dickson (13) found that plants inoculated in the flowering stage were more susceptible to infection than those inoculated in the later stages of development. In order to obtain further information regarding the comparative susceptibility of plants in the flowering, after-flowering, and past-flowering stages of development, 18 pots of wheat were inoculated in each of these stages. Six pots from each set of 18 were placed in each of the 20°, 25°, and 30° C. humidity tents. The results from the 36-hour series are illustrated in figure 2, A. In gen-

TABLE 3.—*Comparative susceptibility of plants in different stages of heading to Gibberella headblight*

Stage of development at inoculation	Heads		Spikelets		
	Inoculated	Infected	Inoculated	Infected	Killed
	Number	*Per cent*	*Number*	*Per cent*	*Per cent*
Boot	23	0	131	0	0
Before flowering	36	53	378	6	4
Flowering	20	100	188	79	84
After flowering	6	100	46	98	87
Past flowering	66	100	549	100	100

eral, the plants inoculated in the past-flowering stage were more susceptible to infection and headblight development than those inoculated in the flowering stage. For example, the series exposed to 36 hours of continued wetness at 25° C. had 54, 68, and 98 per cent of the spikelets infected and 14, 74, and 94 per cent of the spikelets killed in the flowering, after-flowering, and past-flowering stages, respectively.

A difference was noted in the size of the kernels produced on plants inoculated in the various stages and subjected to optimum conditions for infection and blight development. Few or no kernels were produced on those plants inoculated in the flowering stage. The kernels from plants inoculated in the after-flowering and past-flowering stages were progressively larger. The kernels from plants inoculated during and after the flowering stage and up to the dough stage were always small and shrivelled.

The degree of symptom expression varied with the stage of development at the time infection occurred. Heads of wheat infected in the flowering or after-flowering stages were marked by deep brown to slightly reddish or creosote-colored lesions. These symptoms first appeared on the lemma, then on the base of the spikelets, the rachis, and the culm. Heavily infected spikelets, killed as a result of infection, gradually lost the deep
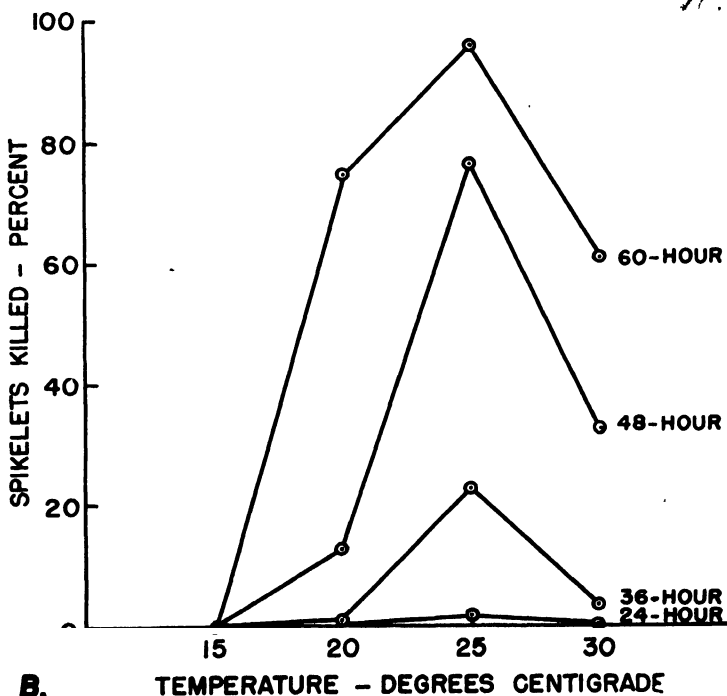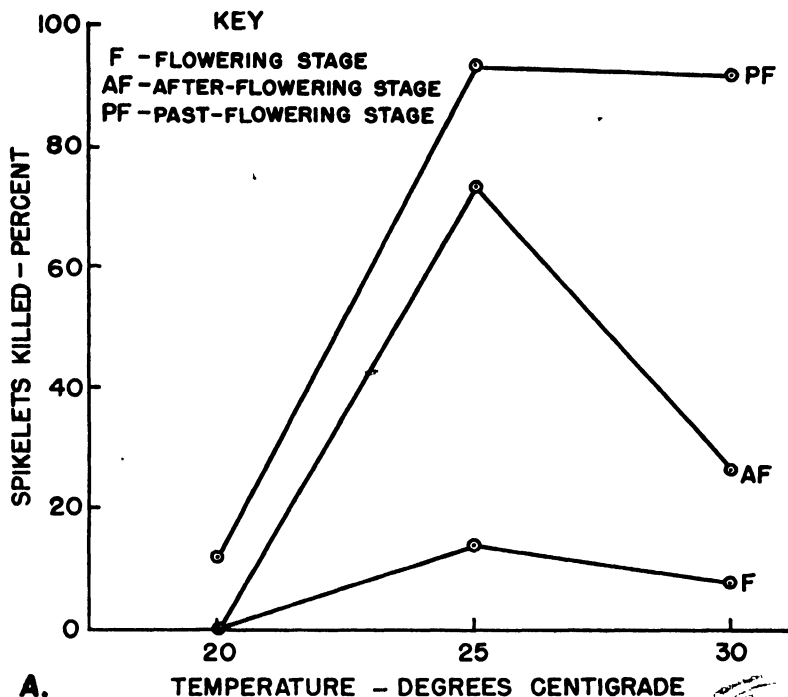
FIG. 2. A. The comparative susceptibility of Newthatch wheat in different stages of development to infection by *Gibberella zeae*. Plants were exposed 36 hours to continued wetness after inoculation. B. The relation of temperature and period of continued wetness to Gibberella headblight. Plants inoculated in the past-flowering stage.

brown discoloration and became straw-colored. Generally, the dark brown discoloration was retained on the culm and rachis. Those plants inoculated in the past-flowering stage of development showed no distinct coloration. Instead, the infected heads and spikelets became bleached and straw-colored.

INFLUENCE OF AIR TEMPERATURE AND MOISTURE ON DISEASE DEVELOPMENT

Infection Studies

Several experiments were conducted in which air temperature and moisture were studied in relation to the infection of wheat heads inoculated with *Gibberella zeae*. Only three of these will be mentioned specifically since the results obtained coincide very closely with those in the other experiments. The experiment on the effect of these factors on the development of headblight of wheat in the flowering, after-flowering, and past-flowering stages of development was mentioned in the preceding section (Fig. 2, A). In the second experiment, 72 pots of wheat in the past-flowering stage of development were inoculated in lots of 8 at regular intervals over a 60-hour period. Two pots from each lot were immediately placed in each of the four humidity tents maintained at 15°, 20°, 25°, and 30° C., respectively. All the pots were removed to the greenhouse bench at the end of the 60-hour period. Thus it was possible to make comparisons of inoculated plants exposed for 6, 12, 18, 24, 30, 36, 42, 48, and 60 hours to continued wetness at the 4 temperatures. The results are illustrated in figure 2, B. In the third experiment, 96 pots of wheat in the flowering stage were all inoculated at one time and distributed equally among the 4 humidity tents maintained at the same temperatures as in the experiment just mentioned. Three pots were removed from each tent after 6, 12, 18, 24, 36, 48, 60, and 72 hours (Table 4).

The period of continued wetness appeared to be an important factor in the determination of the optimum temperature for infection and headblight development. Long exposures at 25° and 30° C. resulted in complete killing of all inoculated wheat heads. Plants given a shorter exposure to continued wetness at 25° C. always developed the most severe headblight. The disease was less severe at 20° than at 25° and 30° C. and only a trace of infection occurred at 15° C.

There appeared to be a definite period during the exposure of inoculated plants to continued wetness at temperatures from 20° to 30° C., in which there was a considerable increase in infection and disease severity. Infection, in this case, is based upon the visible number of infected spikelets, whereas disease severity is based upon the number of spikelets killed. For example, those plants inoculated in the flowering stage and incubated at 25° C. showed an increase in infection from 18 per cent with 36 hours to 77 per cent with 48 hours of exposure, and the disease severity increased from 19 to 64 per cent during this same period. At 20° C. the increase in infection was from 30 per cent with 60 hours to 80 per cent with 72

hours of exposure and disease severity increased from approximately 13 per cent to 96 per cent during the same period (Table 4). Similar results were obtained with plants inoculated in the past-flowering stage (Fig. 2, B). This definite period, which may be referred to as the "critical exposure period" for infection and headblight development, was not so noticeable at 30° C.

TABLE 4.—*Gibberella headblight resulting from exposure of plants, inoculated in the flowering stage, to different periods of continued wetness at different temperatures*[a]

Temperature, degrees C.	Exposure period	Heads	Spikelets		
			Total	Infected[b]	Killed[c]
	Hours	*Number*	*Number*	*Per cent*	*Per cent*
15	18	29	253	0.0	0.0
	24	29	226	0.0	0.0
	36	26	217	0.0	0.0
	48	27	248	1.6	2.0
	60	29	244	0.0	0.0
	72	28	241	0.0	1.2
20	18	29	234	1.3	1.3
	24	28	273	2.9	5.9
	36	27	239	4.2	6.3
	48	27	214	5.2	8.0
	60	28	239	30.0	12.6
	72	27	253	80.6	68.8
25	18	29	212	2.4	6.6
	24	27	237	5.5	2.5
	36	26	232	18.5	19.0
	48	29	267	77.1	64.0
	60	27	243	83.0	76.2
	72	23	223	95.6	84.9
30	18	26	189	0.0	0.0
	24	28	232	0.0	0.0
	36	27	221	2.6	3.2
	48	25	232	27.1	27.1
	60˙	23	196	61.2	29.6
	72	26	213	86.4	....[d]

[a] There were 3 replications for each treatment. No infection resulted after 6 and 12 hours of exposure.
[b] Recorded 6 days after inoculation.
[c] Recorded 12 days after inoculation.
[d] Some plants were killed by foot-rot.

The importance of the interaction of moisture, temperature, and stage of host development to infection and resulting headblight may readily be seen by comparing the results in figure 2, B, with those in table 4. The results from these experiments represent a comparison of the past-flowering and flowering stages of development. Thus, at 25° C. the 60-hour exposed series had 13 per cent more infection and 20 per cent more spikelets killed in the past-flowering than in the flowering stage. With shorter exposures, the differences were less. This is further illustrated in figure 2, A. From these results it appeared that the degree of headblight de-

velopment is dependent upon the period of exposure to moisture, the air temperature, and the stage of host development as well as the quantity of inoculum.

Period of Incubation

Pugh, Johann, and Dickson (13) noted that the length of the incubation period was influenced by temperature and that higher temperatures (32° C.) accelerated the appearance of symptoms. In the present studies, the incubation period was determined by making daily observations from the time the plants were removed from the tents until symptoms became visible (Table 5). Plants inoculated in the flowering stage and exposed for 36 hours at 25° C., showed symptoms in 2 days, whereas plants from the same series which were exposed for 24 hours did not show any sign of infection until the fifth day. Similarly, the 48-hour exposed series at 30° C. had an incubation period of 2.5 days as compared to 6 days for those receiving only 36 hours' exposure. There was a gradual decrease in the length of

TABLE 5.—*Incubation periods for plants inoculated in the flowering stage with Gibberella zeae and exposed to different periods of continued wetness at 15°, 20°, 25° and 30° C.*[a]

Temperature, degrees C.	Hours of exposure					
	18	24	36	48	60	72
	Incubation period—days					
15	.. .	. ..	..	. .		
20	7	6	6	5	4	3
25	6	5	2	2	2 ·	2
30	.		6	2.5	2.5	2.5

[a] Plants were incubated on the greenhouse bench at 25° C. after removal from the humidity tent.

the incubation period for those plants inoculated in the flowering stage and exposed at 20° C. It appears that the length of the incubation period was determined entirely by the establishment of the fungus in the host tissues at the time the plants were removed from the tents and placed on the greenhouse bench, and that temperature and moisture were the controlling factors in the initial establishment of this infection.

Symptom Expression

Aerial mycelium was consistently observed on the surface of the glumes at the time the plants were removed from the humidity tents. In order to determine if there was any relationship between its presence and the length of exposure to continued wetness at various temperatures, records were taken on the presence or absence of mycelium on inoculated wheat heads at the time they were removed from the tents. In general, the amount of aerial mycelium increased with the length of exposure at any one temperature. There was considerably more at 30° than at 25° C., but less at 20°

C. The presence of aerial mycelium on a spikelet was not a sign of infection of that particular spikelet. It was consistently noted that mycelium growth occurred most frequently in degenerating anther tissue. These observations show that temperature and moisture, as well as stage of development, influence the growth of aerial mycelium on the surface of the spikelets.

Differences were also noted in the degree of color expression. Those plants exposed at 30° C. produced the least discoloration. The deepest browning occurred on those plants exposed to temperatures of 20° and 25° C. For those in the latter group, the symptoms appeared to be more pronounced in those plants exposed the longest to continued wetness.

Discontinuous Wetness

To simulate the variable environment occurring under natural field conditions, inoculated plants were exposed to discontinuous or intermittent

TABLE 6.—*Effect on resulting headblight on plants inoculated in the flowering stage with Gibberella zeae, of no exposure and a 6-hour initial exposure to continued wetness at 25° C. followed by 4 and 8 days of drying and a second wetness period of 48 hours at 25° C.*

Exposure period	Drying period	Heads	Spikelets		
			Total	Infected	Killed[a]
Hours	*Days*	*Number*	*Number*	*Per cent*	*Per cent*
0	4	13	111	15.3	10.0
	8	15	150	36.7	15.3
6	4	17	191	21.5	9.4
	8	19	191	33.0	19.9
48	0	15	144	93.0	52.1

[a] Recorded 10 days after inoculation.

wetness in the greenhouse. In one experiment, 10 pots of wheat were inoculated in the flowering stage and treated as follows: (a) Two pots were exposed to continued wetness for 48 hours at 25° C. immediately after inoculation; (b) four pots were exposed 6 hours to continued wetness at 25° C. and (c) four were immediately placed on the greenhouse bench in a relatively dry environment. Four and 8 days later, 2 pots from series b and 2 pots from series c were subjected to 48 hours of continued wetness at 25° C. (Table 6). It was found that drying periods of 4 or 8 days immediately after inoculation or a short wet period of 6 hours followed by a 4- or 8-day dry period resulted in a considerable reduction in infection and disease severity. Identical conclusions were made from the results obtained in two similar experiments.

Sporulation

The preceding sections dealt principally with the initial establishment of infection and subsequent disease development as influenced by temperature and moisture. This section is devoted to a study of the relation

of these factors to sporulation on wheat heads, a feature which is important to the secondary spread of the fungus under field conditions. In the first set of experiments, the plants were inoculated and exposed for 48 hours to continued wetness at 25° C. and then incubated on the greenhouse bench for a week until the symptoms were well developed and some of the spikelets killed. At the end of this period several of the plants were re-exposed to continued wetness for indefinite periods. Wheat heads were removed at regular intervals, washed in water, and conidial counts made to determine the number of conidia present on the heads. The results from one of these experiments are presented in table 7. The second set consisted of plants which were inoculated and placed immediately in the tents for indefinite periods. Heads were removed from these plants at periodic intervals and the number of conidia determined. The results from a representative experiment are presented in table 8. In this way a

TABLE 7.—*Sporulation of Gibberella zeae on infected heads of plants exposed for different lengths of time to continued 'wetness at 15°, 20°, 25° and 30° C. in the humidity tents*[a]

Temperature, degrees C.	Hours of exposure to moisture[b]		
	24	48	72
15	0.00	0.34	2.62
20	0.28	3.10	11.88
25	0.63	4.27	14.11
30	0.31	1.63	5.11

[a] Values represent average number of conidia, in millions, from 3 heads.
[b] No conidia were present on those heads removed after 12 hours' exposure.

good estimate of the number of days required for conidia to be produced and the rate at which they were produced was obtained.

Differences in sporulation were encountered on the heads of wheat in which infection was already well established when they were placed in the humidity tents and exposed to continued wetness for various periods at temperatures of 15°, 20°, 25°, and 30° C. (Table 7). A few conidia were produced after 24 hours of exposure at the three higher temperatures. Conidia were produced in 48 hours on the heads exposed at 15° C. In a previous experiment in which heads were removed from plants after exposures of 12, 18, and 24 hours, conidia were produced only on those removed after 24 hours. The best temperatures for sporulation were 20° and 25° C. At these temperatures an average of 3–4 million conidia was produced on each head in 48 hours and 12–14 million in 72 hours.

Slightly different results were obtained in those studies on sporulation involving plants exposed continuously to moisture from the time of inoculation (Table 8). In this series only the 20°, 25°, and 30° C. temperature tents were used. Initial experiments indicate that at least 3 days would be necessary for conidia to be produced on wheat heads treated in this manner and exposed at any of the above mentioned temperatures. In this

experiment (Table 8) a few conidia were observed at 20° C. 5 days after inoculation, and an average of 5 million conidia per head after 6 days. At 25° C., an average of 36 million conidia per head was produced 6 days after inoculation. Like results were obtained with Cadet wheat plants that received similar treatments. It is evident that a longer time was required for conidia to be produced on plants inoculated and exposed continuously to moisture than on plants which already had disease lesions present on their heads at the time they were subjected to continued wetness. This is probably because the fungus must establish itself in the host tissue before it can begin to reproduce.

To obtain further information on sporulation of *Gibberella zeae* on infected wheat heads under different temperature conditions, such heads were removed from the plants and placed in water. The heads were divided into seven lots and each lot put in a small humidity chamber prepared by

TABLE 8—*Sporulation of Gibberella zeae on heads of wheat inoculated in the flowering stage and exposed to continued wetness at 20°, 25°, and 30° C. in the humidity tents*[a]

Temperature, degrees C.	Hours of exposure to moisture			
	72[b]	96	120	144
20	0.00	0.00	0.15	5.00
25	0.58	1.60	8.19	36.82
30	0.27	1.51	13.44	17.84

[a] Values represent average numbers of conidia, in millions, from 3 wheat heads.
[b] No conidia were present on those heads removed after 48 hours of exposure.

lining a 4000-ml. beaker with moist paper toweling and covering it with a glass plate. The heads were incubated at 4° intervals from 8° to 32° C. Three heads were removed from each set at 12-hour intervals for the first 60 hours and at two subsequent 24-hour intervals. The total number of conidia present on each sample was determined. A few conidia were produced within 48 hours at 24° and 28° C. After 3 or 4 days, numerous conidia were being produced at temperatures from 20° to 32° C., and a few at 16° C. It is evident from these studies that high temperatures favor sporulation as well as infection and disease development.

DISCUSSION

It was apparent that the isolate used in these studies was similar in its temperature requirements to those isolates used by Dickson (4), MacInnes and Fogelman (9), Tu (17), and Tanja (16). Collectively, these authors found that the cardinal temperatures for the growth of *Gibberella zeae* were approximately 3°, 24–28°, and 32–36° C. In the present studies the cardinal temperatures for growth were approximately 4°, 28°, and 32° C., with no growth at 36° C. Further evidence of this agreement was obtained by comparing the results on infection studies obtained by Pugh,

Johann, and Dickson (13) with similar observations made in the present studies.

Many factors enter into a study on the relationship of the environment to disease development. In the present studies on the development of wheat headblight caused by *Gibberella zeae,* some of the more important factors encountered which influenced the amount and severity of disease development were the inoculum dosage, the stage of host maturity, the period of exposure of the plants to a moist atmosphere, and the air temperature at the time of exposure. The factors relating to the variation of the pathogen were not considered in this study, although they, too, are of utmost importance in a study of this type (17). However, all of these factors are sufficiently important to be considered not only in greenhouse experimentation but in field experimentation as well, whether it be by the plant pathologist or the plant breeder.

Inoculum dosage was shown to affect the amount and severity of wheat headblight. In the field this would entirely depend upon the prevalence of the pathogen on crop residues and as a result, it would be affected by the control measures applied. MacInnes and Fogelman (9), Dickson and Mains (5), Muncie (11) and others suggest that Gibberella headblight (scab) can be kept to a minimum by using clean seed, practicing field sanitation, and by using proper crop rotation.

The stage of host maturity at the time of incubation and infection was another factor of considerable importance. This had already been mentioned by Pugh, Johann, and Dickson (13) who noted that wheat was most susceptible to infection in the flowering stage. The results from this study, however, indicated that there was an increase in susceptibility of wheat heads to infection as the plants increased in maturity from the flowering to the past-flowering stage. This discrepancy with previous work may have resulted from differences in the method of incubation or in the method of evaluating infection. Pugh, Johann, and Dickson (13) incubated their plants continuously in chambers maintained at approximately 70 per cent relative humidity and based their conclusions on the greater reduction in kernel weight and on the larger number of infected seeds which they obtained from plants inoculated in the flowering stage. The results presented in this paper were based on the number of infected and killed spikelets. If the seeds from the 36-hour series at 25° C. had been weighed, no doubt those from the flowering stages would have weighed more than those from the past-flowering stage. However, had the 60-hour series at 25° C. been used as a basis for comparison, then higher yields would doubtless have been obtained from those plants inoculated in the past-flowering stage since there were few or no kernels produced on those plants inoculated in the flowering stage. This would indicate that the relative susceptibility of the plants in the different stages of development was dependent upon the length of exposure to continued wetness. However, in all the experiments on Gibberella headblight reported upon in this paper, it was consistently

noted that the plants inoculated in the past-flowering stage were more susceptible than those given a similar treatment in the flowering stage.

Several workers (2, 3, 5, 8, 9, 10, 12, 14, 15) have mentioned that Gibberella headblight of wheat is favored by periods of wet, humid weather and high temperature during the heading period. They did not, however, determine how long an exposure was necessary for infection. Atanasoff (2) and Christensen, Stakman, and Immer (3) further stress the importance of these conditions to sporulation and secondary spread of the organism. The results presented in this paper confirm the observations made by the above authors as to the important part played by moisture and temperature in the development of Gibberella headblight epiphytotics. Not only do these two factors influence the establishment of infection, but they influence the numbers and the rapidity at which conidia are produced on the infected heads. It would be difficult to estimate the actual numbers of conidia that could be produced within 2–3 days in a field of infected wheat under optimum conditions for sporulation and blight development. The fact that the fungus is capable of establishing itself in the host tissue within 24–36 hours at 25° C., and, in addition, is able to produce one-half million new conidia on each infected wheat head within 72 hours, may be a partial explanation of why the organism is capable of spreading rapidly under favorable weather conditions.

Of special significance to those investigators involved in the control of Gibberella headblight by breeding for disease resistance, was the varying amount of infection and severity of headblight development resulting from the exposure of the inoculated plants to different temperature and moisture conditions during different stages of development. Wheat varieties are ordinarily tested for resistance in the field where conditions vary from day to day and from season to season. Very seldom, under such conditions, are all plants in the same stage of development at the time of inoculation and when favorable conditions for infection and headblight development prevail. Therefore, it would be expected that various degrees of infection may be present and that many plants may escape infection entirely. The conclusion of Christensen, Stakman, and Immer (3) that "Considerable caution is necessary in drawing conclusions from the results of varietal tests unless they are conducted for several years under carefully replicated and controlled conditions," is borne out by this work.

SUMMARY

1. The growth of *Gibberella zeae* mycelium and the germination of the conidia took place at temperatures from 4° to 32° C., with the most rapid growth of the mycelium and the elongation of the germ tubes occurring at 28° C. The most rapid germination occurred at 28° and 32° C.

2. The most rapid production of conidia occurred at 28°–32° C. on agar media; but on longer incubation, more conidia were produced at 32° C. than at any other temperature. Few conidia were produced at and

below 16° C. and none were produced at 36° C. Mainly 5-septate conidia were formed at 20°–24° C., whereas 2-septate conidia predominated at 32° C.

3. Conidia were produced on infected heads of plants exposed to continued wetness at temperatures from 15° to 30° C. The maximum number was produced on those heads exposed at 25° C. with high production occurring at 20° C. At 25° C., conidia were produced within 24 hours on plants which already had infection well established at the time the plants were exposed to continued wetness, whereas 72 hours of exposure were required for the formation of conidia on plants exposed to continued wetness from the time they were inoculated. An average of 14 million conidia per head was produced in 3 days after re-exposure of the infected plants and 36 million were produced in 6 days on plants exposed continuously after inoculation.

4. The amount of infection and disease severity increased with an increase in the amount of inoculum up to 1.0–2.0 million conidia per pot of 10 wheat plants.

5. Little infection occurred on wheat heads inoculated prior to flowering. The order of increased susceptibility of wheat to Gibberella headblight was from the flowering to the past-flowering stages, with decreasing susceptibility at late stages of seed development.

6. The best temperature for infection and headblight development was 25° C. Little or no infection occurred at 15° C. More rapid infection occurred at 30° than at 20° C., but after 60–72 hours' exposure to continued wetness the amount of infection and disease development was approximately the same at these two latter temperatures.

7. The period of exposure to continued wetness favoring maximum infection and disease severity varied with the stage of host development and the temperature during exposure. The period of exposure necessary for infection was the shortest at 25° C., becoming progressively longer at the lower and higher temperatures. The exposure period necessary for infection was the shortest (at any one temperature) for those plants inoculated in the past-flowering stage and longest for those inoculated in the flowering stage.

8. The critical exposure periods for disease severity were, for plants inoculated in the past-flowering stage, from 48 to 60 hours at 20° C. and 36 to 48 hours at 25° C.; for plants inoculated in the flowering stage, 60 to 72 hours at 20° C., and 36 to 48 hours at 25° C.

9. Symptoms became visible 2 days after inoculation on plants inoculated in the flowering stage and exposed to continued wetness for 36 hours at 25° C. At 20° and 30° C. with the same exposure period, no symptoms were visible until after 6 days' incubation. In general, longer incubation periods were encountered at the lower and higher temperatures and with shorter periods of exposure to continued wetness at any one of these temperatures.

10. A dry period of 4–8 days immediately following inoculation of plants or following an initial 6-hour exposure of inoculated plants to continued wetness, resulted in a reduction in disease severity in comparison with that obtained on plants receiving no intermediate drying period after inoculation.

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RELATION OF SOIL FUMIGATION, NEMATODES, AND INOCULATION TECHNIQUE TO BIG VEIN DISEASE OF LETTUCE

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There are only a few known plant viruses whose occurrence in nature seems to be intimately associated with the soil. These viruses, or the diseases they cause, are frequently referred to as being soil-borne. The best known virus of this type is probably that causing mosaic of wheat (10). Big vein of lettuce and some oat mosaics (13) are also soil-borne. These diseases seem to have the following characteristics in common: They occur in nature only when susceptible plants are grown in soil containing the causative agent; vectors, if they exist are not known; infested soils can be made non-infective by treatment with formaldehyde or by partial steam sterilization; they are more common in heavy soils than in light soils; the causative agent persists in the soil for long periods of time in the absence of known host plants; infection under natural conditions appears to take place through the roots of susceptible plants.

The suggestion that nemtaodes might be possible vectors of the soil-borne virus causing winter wheat mosaic was made by McKinney (11) and Johnson (7). Johnson (7) found that the symptoms of wheat mosaic did not develop in wheat grown in soil treated with certain fumigants. He suggested that the action of the fumigants was probably on a vector rather than on the virus. McKinney (12) also suggested that wheat-mosaic-infested soil contained some important contributing factor in addition to virus and that it was possible that the virus occurred in some soil-inhabiting organism that served as a vector.

The present investigation was undertaken to ascertain the relationship of nematodes to the transmission of lettuce big vein. Experiments with artificial transmission were undertaken to determine the probable action of fumigants on big-vein-infested soil and to clarify results obtained in nematode transmission tests. Observations were also made on some physical properties of the virus.

REVIEW OF LITERATURE

Lettuce big vein disease was first described by Jagger and Chandler (5) from lettuce grown in the Imperial Valley of California. Jagger and Chandler (5) stated that the causal agent of big vein was apparently soil borne since symptoms of the disease did not develop in plants grown in soil treated with formaldehyde or by partial steam sterilization. It was found that the incidence of the disease was not influenced by the addition of certain chemicals and fertilizers to infested soil. They also reported that the causative agent could persist in soil for long periods of time in the field and in the greenhouse.

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The effect of soil moisture on the development of the symptoms of lettuce big vein disease was investigated by Pryor (14). He found that high soil moisture favored the development of the symptoms in lettuce, and that vigorously growing plants seemed to be most readily affected by the disease. He reported that the earlier a plant developed the disease, the smaller its final size. Thompson and Doolittle (17) observed that lettuce appeared to develop symptoms of big vein disease more readily in air temperatures of 7.2° to 15.5° C. than in air temperatures of 10° to 23.8° C. Pryor (15) found that the optimum soil temperature for the development of big vein symptoms was approximately 22° C. However, he reported that lettuce plants developed symptoms at all soil temperatures ranging from 18° to 30° C.

Rawlins and Tompkins (16) obtained negative results in the artificial transmission of big vein by leaf-rubbing with juice from the foliage of infected plants, with and without carborundum as an abrasive. Pryor (15), reporting the results of investigations conducted in 1941, also obtained negative results in experiments involving artificial transmission of the disease. He concluded from the results of numerous trials that big vein is rarely, if ever, transmitted by the seed of infected plants. Thompson, Doolittle, and Smith (18) were unable to transmit big vein by any of the methods commonly used to transmit plant viruses, but Doolittle and Thompson (2) reported a year later that they had been successful in artificially transmitting lettuce big vein virus. They succeeded in obtaining infection in 56 out of 76 plants inoculated by rubbing leaves or pricking freshly extracted root juice from big-vein-infected plants into the leaves or stems of healthy plants. Parallel inoculations with the juice from the mottled leaves of the plants whose roots were used as inoculum produced no infection. Eighty control plants remained healthy throughout the experiment.

Several insect species have been tested as possible vectors of lettuce big vein disease. Thompson, Doolittle, and Smith (18) obtained no evidence of big vein transmission with *Aphis gossypii* Glover, *Macrosiphum solanifolii* (Ashmead), and *Trialeurodes vaporariorum* (Westwood). A low percentage of transmission was reported using as vectors *Myzus persicae* (Sulzer), *Myzus circumflexus* (Buckton), and an undescribed *Macrosiphum* species. They were apparently able to secure some transmission of lettuce big vein virus with the root aphid *Pemphigus lactucae* (Fitch). It is pointed out by these authors that the presence of big vein disease in the control plants of their experiment might be explained on the basis of the migratory habits of this aphid. Pryor (15) did not obtain consistent transmission of lettuce big vein virus by the aphids, *Macrosiphum solanifolii* (Ashmead), *Myzus solani* Kaltenbach, and *Myzus persicae* (Sulzer). The root aphid, *Pemphigus lactucae* (Fitch), was not present in his greenhouse and he suggested that other vectors might exist.

Soil transmission of lettuce big vein virus has been reported by Jagger and Chandler (5), Thompson, Doolittle, and Smith (18), and Pryor (15).

Pryor (15) prepared leachates from big-vein-infested soil and found that filtering this leachate through a medium Mandler filter removed the causative agent. Unfiltered leachate added to big-vein-free soil produced big vein symptoms in four of 84 plants. That leaching the soil with large amounts of water does not reduce the infectivity of the soil was reported by Jagger and Chandler (5) and Pryor (15). Pryor (15) diluted big-vein-infested soil one part to 800 parts of autoclaved soil and found the incidence of disease was only slightly reduced by the dilution. He reported that air-dry soil remained highly infective for at least eight years.

<center>MATERIALS AND METHODS</center>

The investigations on the transmission of lettuce big vein virus were conducted in the greenhouse and in the laboratory. Big-vein-infested soil was secured from infested lettuce fields in the Salinas Valley. In certain experiments infested soil was mixed with greenhouse soil at a ratio of one to one. This was considered satisfactory procedure since Pryor (15) has reported that such dilutions do not decrease the infectivity of the soil.

Handling of Nematodes. The nematodes tested as possible vectors of big vein virus were collected from soil in which diseased lettuce plants were growing. They were removed from the infested soil by the procedure outlined by Cobb (1). This consisted of mixing soil containing the nematodes with water. The soil-water mixture was passed through a series of graded screens and finally through a piece of finely woven bolting silk. The silk used in these experiments had apertures of 30 to 50 microns in diameter and retained large numbers of the small nematodes present in the soil. The residue remaining in each screen was placed in tap water and examined under a dissecting microscope. Nematodes were removed from the residue by means of a fine bamboo splinter. Each nematode was handled singly to avoid transfer of the debris present in the screening residue. Nematodes were transferred to a small watch glass containing distilled water. After the nematodes to be used in any particular test had been collected, they were transferred to heat-treated, autoclaved or fumigated soil by means of a small pipette. The treated soil was contained in four-inch or six-inch greenhouse pots. After inoculation of the soil with nematodes, the pots were placed on wooden benches in the greenhouse and lettuce seed was planted directly into the soil or a lettuce seedling grown in virus-free soil was transplanted into each pot. Lettuce in these tests was grown in the inoculated soil at least 60 days before the final results were recorded. In several experiments plants were held under observation more than 90 days before they were discarded. In most instances plants were examined daily beginning 20 days after seeding or transplanting of the lettuce for evidence of the development of big vein symptoms.

Securing Virus-free Soil. The soil used in nematode and artificial transmission investigations was treated by one of three methods to make it free from big vein infestation. The first experiments involved the use of

soil that had been heat-treated in a box type electrically heated soil sterilizer. In subsequent experiments the soil was autoclaved at 17 lb. steam pressure for at least four hours or fumigated with one ml. of chlorpicrin per gal. (231 cu. in.) of soil. The latter two methods proved to be more effective in eliminating big vein from the soil.

Methods of Artificial Inoculation. Artificial transmission experiments were undertaken using the carborundum leaf-rubbing method described by Rawlins and Tompkins (16). In some tests root juice containing the virus was rubbed on the leaves of healthy plants with thumb and forefinger. In the more exacting tests the infective juice was rubbed on the leaves, in the presence of carborundum, with a small cotton swab soaked in the infective juice.

Soil Fumigation. Fumigation tests to determine the effect of soil fumigants on big-vein-infested soil were carried out using infested soil from the field. Prior to fumigation the soil was thoroughly mixed and placed in one-gallon jars. The desired amount of fumigant was introduced into the jar with a pipette and the lid of the jar immediately put in place. The soil was retained in the closed jar for a 48-hour period and then transferred to six-inch greenhouse pots. An aeration period of six days was allowed before lettuce seedlings grown in disease-free soil were transplanted into the fumigated soil. In some experiments it was necessary to make slight changes in the procedures outlined above. These are discussed in detail in connection with the particular experiments involved.

TRANSMISSION TESTS WITH NEMATODES

Some nematodes have feeding habits that might be favorable for the transmission of a virus. Nematodes belonging to the families Tylenchidae and Aphelenchidae possess a stylet that is frequently used to pierce plant cells during the feeding process. Most nematode species in the two families have a bulb-like muscular metacorpus located in the median portion of the esophagus. This bulb is equipped with a triradiate valve which serves as a sort of pump to suck out the contents of cells punctured by the stylet. Linford (8) has observed that several nematode species regularly eject secretions of the esophageal glands during the feeding process. This appears to be essentially the same process by which insect vectors are thought to transmit plant viruses. An investigation of the relationship of nematodes to the transmission of lettuce big vein virus was undertaken because of the apparent underground mode of infection, because no previous investigation had been made regarding the possibility that some nematode might be a vector of big vein virus.

Prior to inoculating virus-free soil with nematodes, samples of big-vein-infested soil were examined to determine the nematode species commonly associated with the roots of diseased lettuce plants. The roots of infected lettuce plants were carefully washed free of soil and examined for the presence of nematodes that might be feeding on the roots. In addition some

roots were stained with acid-fuchsin in lacto-phenol following the procedure suggested by Goodey (3). These roots were carefully examined for the presence of nematodes within the root tissues.

The nematode *Paratylenchus macrophallus* (de Man) was the only species observed feeding on the roots of lettuce. For this reason it was given first consideration as a possible vector of lettuce big vein virus. *Aphelenchus avenae* Bastian, a species frequently observed in the roots of plants, was found to be rather abundant in the soil around the roots on infected lettuce. This species was not observed feeding upon the roots or within root tissue. But because of its abundance in the soil *A. avenae* along with *P. macrophallus* was used in the initial transmission studies. *Acrobeloides bütschlii* (de. Man), *Criconemoides mutabile* Taylor, and *Rhabditis monohystera* Bütschli were used separately in subsequent tests. Other nematode species found in

Table 1.—*Number of lettuce plants developing big vein symptoms when grown in heat-treated and autoclaved soil inoculated with nematodes taken from infective soil, in heat-treated soil, autoclaved soil, and in soil infested with big vein*

Treatment	Number of nematodes per plant	Number of plants inoculated	Number of plants infected
Paratylenchus macrophallus	50	10	5[a]
do.	100	29	0
Aphelenchus avenae	20	10	0
Acrobeloides bütschlii	50	20	0
do.	100	20	0
Rhabditis monohystera	100	10	0
Criconemoides mutabile	16	10	0
Miscellaneous nemas	50	20	1[a]
do.	100	20	3[a]
Oligocheat worms	5	20	0
Heat-treated soil		10	2[a]
Autoclaved soil		40	0
Infested soil		34	33

[a] Source of infection uncertain, possibly inadequate heat treatment.

infested soil were not isolated as a single species but were placed in the disease-free soil as miscellaneous lots of nematodes. The following species were present in these lots: *Aphelenchoides parietinus* (Bastian), *Acrobeloides bütschlii* (de Man), *Chiloplacus* sp., *Dorylaimus obscurus* Thorne, *Dorylaimus simplex* Thorne, *Dorylaimus monohystera* de Man, *Panagrolaimus subelongatus* (Cobb), *Rhabditis monohystera* Bütschli, and *Tylenchus filiformis* Bütschli. A few larvae of the sugar-beet nematode, *Heterodera schachtii* Schmidt, were also present in these lots. The inoculation experiments with nematodes were repeated several times and the results are summarized in table 1.

The evidence of nematode transmission of the virus causing big vein of lettuce is mostly negative in these experiments. In no instance was it possible to consistently demonstrate nematode transmission. The relatively few cases of apparent nematode transmission were probably the result of

inadequate heat-treatment of the soil prior to inoculation or to outside sources of infection.

Successful artificial transmission of lettuce big vein virus has been reported only by Doolittle and Thompson (2). Rawlins and Tompkins (16), Thompson, Doolittle, and Smith (18), and Pryor (15) have reported negative results when they inoculated healthy lettuce plants with the juice extracted from the leaves of infectĕd plants. In order to corroborate the results reported by Doolittle and Thompson (2), 20 lettuce seedlings grown in autoclaved soil were inoculated. Freshly extracted root juice of lettuce plants infected with big vein virus was rubbed with the thumb and forefinger, in the presence of carborundum, on the leaves and cotyledons of the seedlings. Twenty lettuce seedlings grown in autoclaved soil were transplanted into big-vein-infested soil and 20 into autoclaved soil. Nine of the 20 inoculated seedlings became infected with big vein virus and 19 of the 20 seedlings transplanted into infested soil developed symptoms. All of the 20 seedlings transplanted into autoclaved soil remained healthy.

The results obtained in the experiment appeared to confirm those reported by Doolittle and Thompson (2), since symptoms of big vein did not develop in the plants growing in autoclaved soil. There was, however, a considerable difference between the incubation period of about 79 days for the inoculated plants in the present experiment and the incubation period of 45 days reported by Doolittle and Thompson (2). It does not appear probable that this difference in incubation time can be attributed to variation in the environmental conditions of the experiments inasmuch as the average incubation period of 40 days for plants grown in big-vein-infested soil is close to the incubation period of 45 days reported by Doolittle and Thompson (2) for lettuce grown in big-vein-infested soil.

The long period of time required for leaf-inoculated lettuce plants to develop symptoms (79 days) gave rise to doubt as to the actual avenue of entrance of the virus into the plants. In this experiment no precautions were taken to prevent contact of the inoculated foliage with the autoclaved soil in which the plants were growing. The leaves of the inoculated plants were not washed after they had been rubbed with the infective root juice and carborundum. However, the plants were irrigated by pouring water on the surface of the soil. This could have served to wash virus present on the external surfaces of inoculated leaves into the soil. In order to obtain more exact information concerning the path by which the virus actually entered the leaf-inoculated plants, additional experiments on artificial transmission were undertaken.

One hundred twenty-five six-inch greenhouse pots were filled with soil and autoclaved for five hours at 17 lb. steam pressure. The pots were then placed in the greenhouse on benches previously drenched with a five per cent

formaldehyde solution. Lettuce seed was planted in the autoclaved soil and after germination the lettuce was thinned so that two seedlings remained in each pot. When these seedlings reached the four-leaf stage, one plant in each of 75 pots was inoculated by rubbing the leaves, in the presence of carborundum, with root juice freshly extracted from big vein lettuce plants. The soil in another lot of twenty pots was inoculated by pouring two ml. of the infective juice onto the surface of the soil. The remaining 25 pots, each containing two plants, were retained as control plants to check the efficacy of the autoclaving treatment. The results of this experiment are given in table 2.

The results clearly demonstrate that the virus causing big vein of lettuce can be transmitted by pouring infective root juice extracts on the soil. Forty-eight out of 50 lettuce plants grown from seed in autoclaved soil that was inoculated with two ml. of infective root juice developed big vein symp-

TABLE 2.—*Number of lettuce plants grown in autoclaved soil developing big vein symptoms as the result of leaf-inoculation and soil inoculation with infective root juice extracted from big vein infected lettuce plants*

Treatment	Number of plants	Number of plants infected	Days for symptoms, average
Autoclaved soil[a]			
Leaf-inoculated	75	17	53.6
Not inoculated	75	11[b]	58.0
Autoclaved soil	50	0	... ...
Autoclaved soil 2 ml. juice poured in each pot	50	48	47.3

[a] Two plants per pot, one plant inoculated, the other not inoculated.
[b] Six of these were the only ones of the pair to become infected.

toms. Since lettuce seed was planted directly in the pots containing the autoclaved soil, the virus seems to have gained entrance into the plants in the absence of wounds other than those that might occur naturally during the growth of the lettuce. None of the plants grown from seed planted in untreated autoclaved soil developed big vein symptoms.

In the test in which one plant per pot was inoculated by leaf-rubbing and the other plant was not inoculated, the results obtained can be explained on the basis of infection through the soil. Seventeen of the 75 plants inoculated by leaf-rubbing developed big vein symptoms and 11 of the 75 plants that were not inoculated but grown in the same pots became infected. The average incubation period for the inoculated plants was about 53 days as compared to 58 days for the plants that were not inoculated. In some instances noninoculated plants in pots with inoculated plants developed symptoms before they appeared in the leaf-inoculated ones. Also, in six of the pots only the plant that was not leaf-inoculated developed symptoms. The incubation period was only 47 days where the inoculum was poured into the soil. Presumably this method supplied more inoculum to the roots in a shorter period of time.

The data presented in table 2 indicate that infection with big vein virus through the foliage of lettuce plants is questionable. The most logical explanation for the results seems to be that infection did not develop as the result of leaf-inoculation, but as the result of the virus used in leaf-inoculations gaining access to the soil and infecting the plants by way of the roots. However, since the foliage of the lettuce plants was not protected from the inoculated soil, the possibility of infection through the leaves from contact with the soil was not eliminated. For this reason the experiment was repeated with methods that would prevent foliage contact with the soil.

Forty four-inch greenhouse pots were filled with soil previously fumigated with chlorpicrin at the rate of one ml. per gal. of soil. Lettuce seedlings grown in similar fumigated soil were transplanted into these pots. A cotton pad one-half inch thick was then placed over the surface of the soil contained in each pot so as to prevent contact of the leaves with the soil. This pad was retained in place throughout the experiment. The lettuce

TABLE 3.—*Number of lettuce plants developing big vein symptoms as the result of controlled leaf-inoculation, leaf-inoculation followed by washing of the plants, soil inoculation with five milliliters of infective root juice, and no treatment*

Treatment	Number of plants inoculated	Number of plants infected
Leaf inoculation (soil covered)	40	0
Leaf inoculation (soil not covered, plants washed)	36	31
Soil inoculation with 5 ml. juice (foliage protected)	20	19
Control	20	1

seedlings in this series were inoculated by rubbing the leaves, in the presence of carborundum, with a small cotton swab soaked in infective root juice. These inoculations were carefully made so that an excess of the inoculum was not used. After inoculation of the plants, the pots were placed in saucers and irrigation throughout the experiment was by pouring water into these saucers. Another forty plants were similarly inoculated. One hour after inoculation these plants were washed with a fine spray of water to remove the carborundum residue from the leaves. The plants were washed in a position that allowed the water to drip from the leaves into the soil. The plants in this series were irrigated by pouring water onto the soil in the pots. Twenty lettuce seedlings growing in fumigated soil were inoculated by adding five ml. of infective root juice to the soil surrounding the opening in the bottom of the greenhouse pots. These plants were irrigated from the bottom by water poured into saucers containing the pots. Prior to inoculation the surface of the soil in these pots was covered with a cotton pad to prevent foliage contact with the soil. Twenty seedlings growing in fumigated soil

were retained as controls. The infective root juice used in this experiment was extracted from the roots of 20 lettuce plants showing symptoms of big vein infection. A portion of this juice was used as inoculum in each of the tests in the experiment. The results of this experiment are in table 3.

The results demonstrate that infection with lettuce big vein virus did not take place through the foliage of leaf-rubbed plants and that unless precautions are taken to prevent the inoculum from dripping or washing into the soil, root infection may be thought to be leaf infection. Two methods of soil inoculation are demonstrated, from above and from below, to be effective in experimentally producing the disease.

INFECTION FROM CHOPPED LETTUCE ROOTS ADDED TO THE SOIL

In 1934 Jagger and Chandler (5) reported that lettuce big vein disease was gradually becoming more serious and wide-spread as the result of continuous cropping of the same land with lettuce. Persistence of big vein virus in the soil probably indicates that the virus is returned to the soil as the roots of infected lettuce plants decompose in these fields. It has been demonstrated that the virus of lettuce big vein can enter the plant as the result of root contact with virus in the soil. In order to determine if big vein virus could be introduced into virus-free soil by the roots of infected lettuce plants, a series of pots containing chlorpicrin-fumigated soil were inoculated with thoroughly washed chopped roots of infected plants. The fumigated soil in each of 10 four-inch pots was inoculated with five gm. of infected lettuce roots. Ten pots containing fumigated soil were retained as controls. Ten pots containing untreated soil from the field were inoculated with five gm. of infected roots and 10 pots containing the field soil received no root material. Ten out of 10 lettuce plants grown in fumigated soil inoculated with infected roots developed big vein symptoms. No big vein symptoms were observed in plants grown in the fumigated soil. Eight of 10 plants grown in field soil inoculated with infected lettuce roots developed big vein symptoms. None of the 10 plants grown in noninoculated field soil developed symptoms. These results indicate that virus-free soil can be inoculated with big vein virus by the addition of big vein infected lettuce roots.

SOIL FUMIGATION

Early in the investigation of the possible relationship of nematodes to the transmission of lettuce big vein virus some preliminary experiments were undertaken involving the fumigation of big-vein-infested soil with a nematicide. Approximately one gal. of big-vein-infested soil was exposed for 48 hours in a sealed container to the fumes of dichloropropene-dichloropropane mixture.[2] One ml. of the fumigant was applied per gal. of infested soil. This amount of the fumigant is about the equivalent of 1,000 lb. per acre. After the 48-hour exposure period, the treated soil was transferred to eight

[2] Shell DD, supplied by the Shell Oil Company.

four-inch greenhouse pots and allowed to aerate for six days. Untreated big-vein-infested soil from the same source was placed in six four-inch greenhouse pots. Six days after the fumigation treatment, lettuce seed was planted directly in the soil. In the fumigated soil one out of eight plants developed symptoms of big vein infection. In the untreated big-vein-infested soil, five out of six lettuce plants developed symptoms. The results obtained in this preliminary test indicated that the fumigant was capable of at least partially inactivating the causal agent of big vein of lettuce.

Johnson (7) reported that carbon disulphide, chlorpicrin, methyl bromide, rotenone, and naphthalene prevented the development of the symptoms of winter wheat mosaic in susceptible wheat grown in soil treated with these chemicals. Calcium cyanide and ethylene dichloride gave incomplete control under similar conditions. Johnson (7) suggested that the action of these fumigants was on a vector rather than on the virus. McKinney (10), McKinney, Webb, and Dungan (9), Webb (19), Ikata and Kawai (4), and Johnson (6, 7) have demonstrated that winter wheat mosaic occurs in nature only when susceptible wheat is grown in mosaic-infested soil. Jagger and Chandler (5), Thompson, Doolittle, and Smith (18), and Pryor (15) have obtained evidence that big vein of lettuce also results from root contact with infested soil. It has been shown in the present paper in the section dealing with artificial transmission that a vector is not necessary for the transmission of the virus. It is therefore probable that the action of the dichloropropene-dichloropropane mixture was on the virus *per se*.

To obtain further information on the effect of fumigation on soil infested with big vein virus, additional experiments were undertaken. Dichloropropene-dichloropropane mixture and 20 per cent by weight ethylene dibromide mixture[3] were applied to virus-infested soil at dosage rates of 0.23, 0.46, 0.69, 0.92, and 1.16 ml. per gal. of soil. The soil was treated in one-gallon jars sealed with gas-tight lids. After the proper amount of fumigant was applied to the soil, the jars were closed for 48 hours. The soil was then removed from the jars and placed in six-inch greenhouse pots. Each gallon jar contained enough soil to fill two six-inch pots. Since five jars of soil were treated at each dosage rate, this afforded 10 pots for each dosage applied. Twenty six-inch pots containing untreated virus-infested soil were included in the experiment as controls. Lettuce seedlings grown in autoclaved soil were transplanted into the treated and untreated soil six days after the fumigated soil was removed from the fumigation jars. The results obtained in this experiment are in table 4.

The results indicate that 20 per cent ethylene dibromide mixture had no effect on the development of big vein symptoms in lettuce planted in fumigated soil. The symptoms of the disease developed just as rapidly in soil fumigated with this material as in untreated virus-infested soil; approximately 30 days were required in both instances. Dichloropropene-dichloropropane mixture had a retarding effect on the development of symptoms at

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[3] Dowfume W–10, supplied by the Dow Chemical Company.

TABLE 4.—*Number of lettuce plants developing big vein symptoms in virus-infested soil treated with various dosages of dichloropropene-dichloropropane mixture and 20 per cent by weight ethylene dibromide mixture*

Milliliters of fumigant per gallon of soil	Treatments					
	Dichloropropene-Dichloropropane			Ethylene dibromide		
	Number of plants	No. of plants infected	Days for symptoms, average	Number of plants	No. of plants infected	Days for symptoms, average
0.23	10	10	52.8	10	10	31.3
0.46	10	1	73.0	10	10	32.5
0.69	10	0		10	9	29.0
0.92	10	1	74.0	10	10	29.1
1.16	10	0		10	10	30.6
No treatment	20	19	28.4			

the lowest dosage applied. At dosages above 0.23 ml. per gal. of soil, only two plants of 40 developed big vein symptoms in soil treated with this material.

In another experiment big-vein-infested soil collected in the Salinas Valley, California, was treated with several fumigants. The method of fumigation was the same as in the previous experiment with fumigants. The following materials were applied to the big-vein-infested soil: Two dichloropropene-dichloropropane mixtures, ethylene dibromide mixture con-

TABLE 5.—*Number of lettuce plants developing big vein symptoms when grown in virus-infested soil treated with various fumigants*

Treatments	Ml. or gm. of fumigant per gallon of soil	Number of plants	Number of plants infected	Days for symptoms, average	Weight of tops in grams, average
Dichloropropene-dichloropropane[a]	0.23	10	8	40.1	22.5
	0.46	10	0		31.7
	0.69	10	0		23.8
Dichloropropene-dichloropropane[b]	0.23	10	8	35.5	21.9
	0.46	10	0		35.0
	0.69	10	0		24.7
Ethylene dibromide[c]	0.23	10	10	29.9	18.5
	0.46	10	10	29.2	21.6
	0.69	10	7	29.1	16.5
Chlorpicrin[d]	0.23	10	0		33.3
	0.46	10	0		47.6
	0.69	10	0		32.5
Benzene hexachloride[e]	0.70	10	10	27.2	17.1
	1.40	10	9	34.5	22.8
	2.80	10	7	30.8	10.0
No treatment		13	13	30.6	

[a] Supplied by the Shell Oil Company.
[b] Supplied by the Dow Chemical Company.
[c] 40 per cent by weight supplied by the Dow Chemical Company.
[d] Supplied by the Innis, Speiden, and Company.
[e] Dust containing one per cent gamma isomer supplied by California Spray Chemical Company.

taining 40 per cent of ethylene dibromide by weight, chlorpicrin, and benzene hexachloride dust containing one per cent of the gamma isomer. The dichloropropene-dichloropropane mixtures, chlorpicrin, and ethylene dibromide mixture were applied to the big-vein-infested soil at the rate of 0.23, 0.46, and 0.69 ml. per gal. of soil. The benzene hexachloride dust was applied at the rate of 0.70, 1.40, and 2.80 gm. per gal. of soil. This amount is approximately the equivalent of 5, 10, and 20 lb. of the gamma isomer per acre. The results of this test are in table 5.

The data recorded indicate that chlorpicrin was the most effective of the fumigants tested in inactivating the virus of lettuce big vein in the soil. The results obtained with dichloropropene-dichloropropane mixtures are similar to those obtained in the previous experiment. Ethylene dibromide and benzene hexachloride did not appear to have any effect on the virus in the soil. Lettuce seedlings grew more vigorously and produced larger plants in the soil that was treated with chlorpicrin and the intermediate dosages of dichloropropene-dichloropropane mixtures. Plants grown in virus-infested soil treated with fumigants that prevented the development of big vein symptoms, or delayed the appearance of symptoms, were larger than plants grown in virus-infested soil treated with materials that did not prevent or delay the development of symptoms. The higher dosages, however, appeared to have inhibited the growth of lettuce.

It was assumed in the previous experiments with soil fumigants that the action of the fumigant was on the virus. This assumption is based on the fact that lettuce growing in autoclaved soil becomes infected with big vein when root juice extracts from big-vein-infected plants are added to the soil in the absence of probable vectors. One attempt was made to secure direct evidence for this assumption. Eighty ml. of root juice were extracted from the roots of big-vein-infected lettuce plants. This juice was diluted with an equal quantity of water and centrifuged for one hour at a speed of 3,000 revolutions per minute. After the centrifugation, the supernatant was filtered through a medium Mandler filter. The filtered juice was added to greenhouse soil previously autoclaved for five hours at 17 lb. steam pressure. The filtered, centrifuged juice did not cause the development of big vein symptoms in lettuce seedlings planted in the inoculated soil. Pryor (15) found that water extracts of big-vein-infested soil became non-infective when filtered through a medium Mandler filter.

THERMAL INACTIVATION OF THE VIRUS

This phase of the investigation was originally intended to be a part of the section dealing with nematode transmission tests. However, experiments with artificial transmission have indicated that big vein virus is free in the soil and it is probable that the results obtained by heating the soil at various temperatures apply to the thermal inactivation point of the virus. Since it is possible to kill all of the nematodes in big-vein-infested soil by a moderate amount of heating, it was the writer's original intention to determine

if the temperature at which nematodes were killed coincided with the temperatures required to make big vein soil non-infective. Preliminary tests indicated that the nematodes present in the soil were killed by heating the soil at a temperature of 45° C. for one-half hour. A few nematodes survived a temperature of 40° C. for one-half hour.

Ten 30-gram samples of big-vein-infested soil containing nematodes were exposed for one-half hour at each of the following temperatures: 35, 40, 45, 50, 55, 60, 65, 70, and 75° C. Each 30-gram sample was placed in a test tube with enough water to wet the soil thoroughly. The tubes containing the soil were then suspended in a water bath heated to the appropriate temperature. After the samples of soil had been heat-treated, the soil was removed from the test tubes and added as inoculum to four-inch pots containing soil that had been fumigated with chlorpicrin at the rate of one ml. per gal. of soil. Ten 30-gram samples of the same lot of soil that had received no heat treatment were placed as inoculum in each of ten pots containing fumigated soil to check on the presence of big vein virus in the soil used in the heat treatment tests. Lettuce seedlings grown in chlorpicrin-fumigated soil were transplanted into the pots containing the inoculated soil. The number of lettuce plants developing symptoms of the ten inoculated with soil heated at various temperatures was: No heat treatment, 9; 35° C., 8; 40° C., 10; 45° C., 7; 50° C., 8; 55° C., 1; 60° C., 1. Big vein did not develop where the soil had been heated above 60° C. As indicated previously, it is extremely doubtful that nematodes could play a part in the transmission of the virus since they are all killed at temperatures which did not inactivate the virus.

In addition to the heat treatment of big-vein-infested soil, 100 lettuce seedlings were inoculated with heat-treated juice from the roots of big vein plants. Prior to inoculation the juice was diluted with an equal volume of water. Ten-ml. portions of this mixture were exposed for one-half hour to temperatures ranging from 30° to 75° C. A similar quantity of the same diluted juice was not exposed to heating and was used to check the infectivity of the extracted juice. Ten lettuce seedlings growing in chlorpicrin-fumigated soil were inoculated with the heat-treated juice, five by leaf-rubbing, and five by pouring the juice on the surface of the soil in which the seedlings were growing. The results obtained in this experiment were very similar to those obtained by heating the virus-infested soil. Complete inactivation of the virus occurred at 65° C. The number of plants developing symptoms as the result of pouring inoculum on the soil was: No heat treatment, 4; 30° C., 5; 35° C., 3; 40° C., 5; 45° C., 4; 50° C., 0; 55° C., 1; 60° C., 1. The number of plants developing big vein symptoms as the result of leaf-rubbing with the inoculum was: No heat treatment, 0; 30° C., 1; 35° C., 2; 40° C., 0; 45° C., 0; 50° C., 0; 55° C., 1; 60° C., 0. It has been demonstrated previously that infection does not result from leaf inoculation but takes place through the roots as the result of soil contamination. This

probably accounts for the low percentage of infection obtained in the leaf-rubbing tests in this experiment.

<div align="center">INFECTIVITY OF DILUTED ROOT JUICE</div>

Infective root juice diluted with various amounts of distilled water was added to chlorpicrin-fumigated soil in which lettuce seedlings were growing. Two ml. of diluted juice was poured on the surface of the soil in each pot. Ten plants were inoculated at each dilution. The dilutions tested ranged from equal parts of juice and water to one part of juice to 200,000 parts of water. The number of plants developing big vein of the 10 inoculated at each dilution was: 1–1, 9; 1–100, 8; 1–1000, 6; 1–10,000, 6; 1–20,000, 7; 1–50,000, 6; 1–100,000, 4; 1–200,000, 3. The dilution end point of the virus in root juice extracts appears to exceed one to 200,000.

<div align="center">DISCUSSION</div>

The present investigation has shown that infection of lettuce plants with big vein virus can take place in the absence of a vector. The virus appears to be free in the soil or possibly absorbed on soil particles. Infection appears to occur as the result of root contact with soil containing the virus. The presence of some sort of wounds is probably a prerequisite for infection, but deliberate wounding of the roots does not appear to be necessary. It is probable that infection can occur through natural wounds such as those caused by abrasion during root growth or the breaking of small roots as the result of alternate wetting and drying of the soil. Symptoms of big vein infection appear to develop more rapidly in plants transplanted into virus-infested soil than in seedling lettuce plants grown from seed in infested soil. This is probably an indication that the wounds produced on the roots of lettuce seedlings during transplanting afford a favorable point of entrance for the virus.

The virus of lettuce big vein probably persists in the soil of infested lettuce fields as the result of the continued addition of new virus to the soil from the disintegrating roots of infected lettuce plants. If this is the case, it would explain the observation of Jagger and Chandler (5) that lettuce big vein disease becomes more abundant and greater crop losses result when lettuce is grown year after year in infested fields. The virus can remain infective when diluted by large volumes of water. This is probably an important factor in the persistence of the virus in cultivated fields.

It has been reported (2) that it is possible to transmit big vein virus by artificial inoculation of the foliage of healthy lettuce plants with freshly extracted juice from the roots of big vein infected plants. Results obtained in the present investigation indicated that infection through the foliage of the plant did not occur when infective root juice was rubbed, in the presence of carborundum, on the leaves of healthy lettuce plants. It was found that the symptoms of big vein infection that were obtained by leaf-rubbing with infective root juice developed as the result of soil contamination with the virus contained in the inoculum used to rub the leaves. It is believed prob-

able that the positive results reported by Doolittle and Thompson (2) to have occurred from leaf-rubbing with infective root juice were due to soil contamination. Washing the foliage of artificially inoculated plants immediately after rubbing the infective juice on the leaves apparently carries the inoculum into the soil.

Winter wheat mosaic and winter oat mosaic are caused by soil-borne viruses that are reported to be transmissible by artificial inoculation of the foliage. However, these viruses appear to differ from lettuce big vein virus in that they are present in the foliage of infected plants (6, 13) while in the case of lettuce big vein virus, Rawlins and Tompkins (16), Thompson, Doolittle, and Smith (18), and Pryor (15) have obtained negative results in transmission experiments involving the use of juice extracted from the foliage of infected plants. For this reason it is doubtful that direct comparison of lettuce big vein virus and the viruses causing winter mosaics of wheat and oats can be made. But it should be pointed out that unless special methods are employed to prevent the possibility of soil contamination with the inoculum, the results obtained by leaf inoculation may be misleading if the virus is one that causes symptoms as the result of root infection.

The fact that treating winter-wheat-mosaic-infested soil with a fumigant such as chlorpicrin made the soil non-infective has been cited as evidence of the presence of a vector in mosaic-infested soil (7, 12), the suggestion having been made that the action of the fumigant was probably upon a vector and not on the virus *per se*. Experiments conducted during the present investigation indicate that lettuce big vein virus is inactivated when virus-infested soil is fumigated with either chlorpicrin or dichloropropene-dichloropropane mixture. It is therefore possible that winter wheat mosaic virus can also be inactivated directly by soil fumigation. If this is the case, it is quite possible that a vector is not necessary for the transmission of the viruses causing winter wheat mosaic and winter oat mosaic.

· SUMMARY

Negative results were obtained in experiments on nematode transmission of big vein virus with the following species of nematodes: *Aphelenchoides parietinus* (Bastian), *Aphelenchus avenae* Bastian, *Acrobeloides bütschlii* (de Man), *Chiloplacus* sp., *Criconemoides mutabile* Taylor, *Dorylaimus monohystera* Bütschli, *Dorylaimus obscurus* Thorne, *Dorylaimus simplex* Thorne, *Panagrolaimus subelongatus* (Cobb), *Paratylenchus macrophallus* (de Man), *Rhabditis monohystera* Bütschli, and *Tylenchus filiformis* Bütschli.

Transmission of the virus through the foliage of lettuce plants did not take place when the leaves of healthy lettuce seedlings were rubbed, in the presence of carborundum, with freshly extracted root juice of infected lettuce when precautions were taken to prevent soil contamination with the inoculum. Evidence was obtained indicating that infection of lettuce with big vein virus occurs only through the roots. Cases of infection that ap-

peared to indicate transmission by leaf-rubbing were demonstrated to be the result of soil contamination with the infective juice used as inoculum. Big vein symptoms developed in lettuce grown in virus-free soil inoculated with juice extracted from the roots of big vein plants and in virus-free soil inoculated with chopped roots of infected plants. Lettuce plants appeared to have developed big vein symptoms as the result of root contact with soil containing the virus and in the absence of any probable vector or deliberate wounding of the roots.

The virus in the soil or in root juice extracts is inactivated when exposed for one-half hour to a temperature between 60° and 65° C. The virus is also inactivated by fumigation of infested soil with chlorpicrin at 0.23 ml. or dichloropropene-dichloropropane mixture at the rate of 0.46 ml. per gal. of soil. Ethylene dibromide mixture and benzene hexachloride did not inactivate the virus. The virus retains its infectivity when freshly extracted root juice is diluted at least one to 200,000 with water.

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HETERO– AND HOMO–THALLIC TYPES OF
DIAPORTHE ON SOYBEANS[1]

A. W. WELCH AND JOSEPH C. GILMAN

The discovery by Welch (9) that two distinct types of perithecial development of *Diaporthe* occurred on overwintered stems of soybeans raised the question of their identity, since up to this time the only Diaporthe species known on this host was *Diaporthe phaseolorum* var. *sojae* described by Lehman (5). One type (Fig. 1) had perithecia borne singly in an effuse stroma while the other (Fig. 2) had perithecia clustered in a caespitose head. Further, the single perithecial type was heterothallic, the caespitose type, homothallic. The heterothallic type was a weak parasite occurring on maturing plants while the homothallic type was a wound parasite causing cankers and girdling on actively growing hosts. Proof of heterothallism in this genus and the identification of the types are the subject of this paper.

In 1923 Lehman (5) described a species of *Diaporthe* as the cause of the pod and the stem blight of soybean, naming it *Diaporthe sojae*. He reported perithecia only from cultures, but later Wolf and Lehman (10) reported them from overwintered stems. At that time speciation in this genus was based largely on host relationships. In 1933 Wehmeyer (8) in his monograph of the genus, changed the emphasis to morphological considerations and reduced Lehman's species to varietal rank under *D. phaseolorum* (Cke) Ell., thus bringing the soybean fungus into close association with the *Diaporthe* described by Harter (2) on Lima beans. Wehmeyer separated this species from *D. arctii* (Lasch) Nit. on the basis of larger spores, larger perithecia, and shorter ostiolar necks in the latter species. He placed certain forms from Europe on *Medicago* and *Melilotus* in *D. arctii* chiefly on the length of their ostiolar necks. In 1935, Tucker (7) reported *Diaporthe phaseolorum* as the cause of fruit rot of pepper. His cultures were proved homothallic by means of single ascospore isolations. Both alpha and beta spores were present in the cycle. He considered his fungus to be close to the variety of the species recognized by Wehmeyer as var. *batatatis*. More recently Luttrell (6) has reported on the host range of *D. phaseolorum* var. *sojae* and *D. phaseolorum* and showed distinct differences in their pathogenicity toward certain hosts. The variety proved to be a weak parasite and attacked a wide range of plants that had reached a moribund condition. *D. phaseolorum* was greatly restricted. Although Luttrell found both perithecial and nonperithecial strains within the cultures he examined, he did not work with single ascospore isolates.

MATERIALS AND METHODS

During March, April, and May of 1946 the single and caespitose peri-
thecial types were found repeatedly on overwintered soybean stems collected
from the field.   Isolations were made from each type by transferring ex-
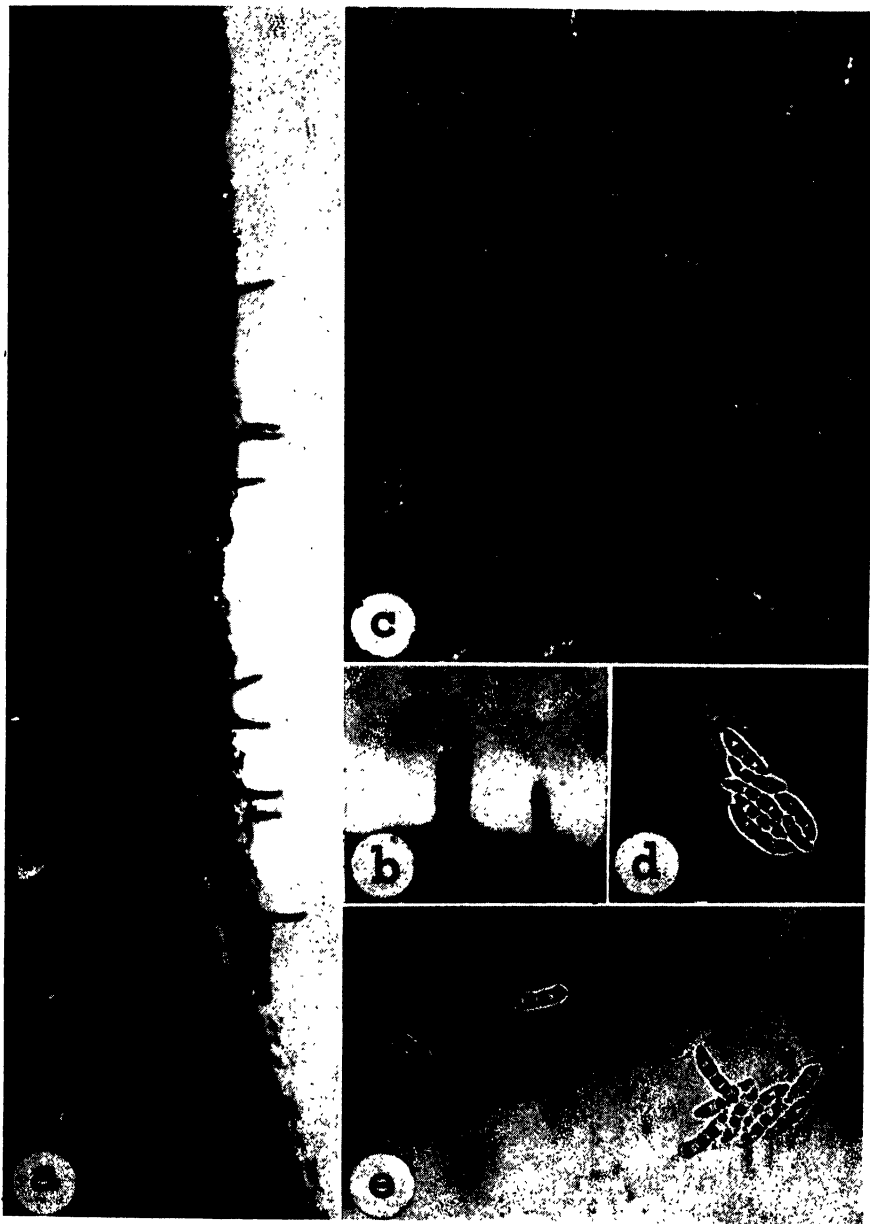
FIG. 1.   *Diaporthe phaseolorum* var. *sojae.*   a. Perithecia on overwintered soybean
cane from the field.   b. Perithecial necks showing ascospore discharge on tips.   c. Alpha
and beta conidia.   d. Ascus and ascospores.   e. Ascospores.

truded ascospores from perithecial beaks to potato-dextrose agar plates. Cultures and sub-cultures obtained from the single perithecial type produced pycnidia when grown on various agar media and steamed soybean stems
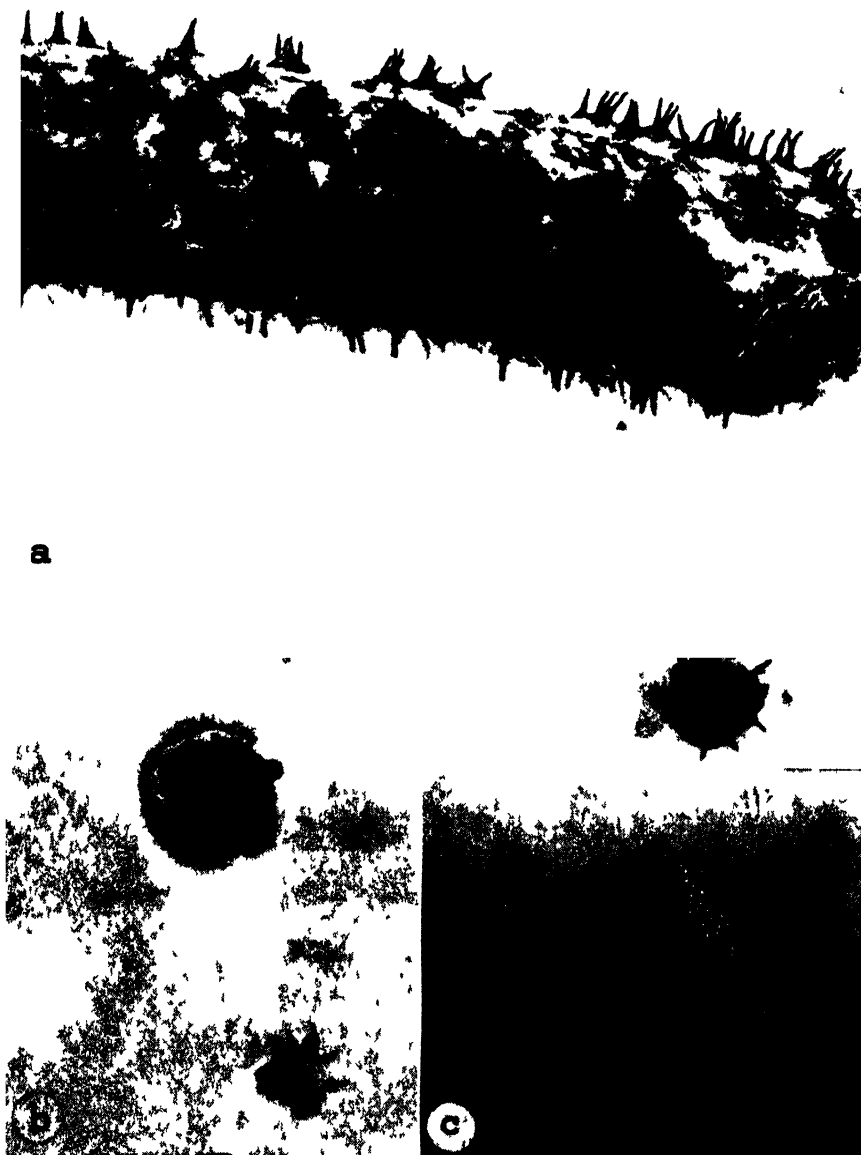
FIG. 2. *Diaporthe phaseolorum* var. *batatatis.* a. Perithecia on overwintered cane from the field. Collected at Ames, Iowa, May 24, 1946. b. Clusters of perithecia on potato dextrose agar plate. c. Ascus and ascospores grown in culture.

FIG. 3.   Cultural characteristics of *Diaporthe phaseolorum* var. *sojae* and *D. phase-olorum* var. *batatatis* on steamed soybean stems.   a. Perithecial beaks of *D. phaseolorum* var. *batatatis*.· b. Pycnidia and conidial exudate of *D. phaseolorum* var. *sojae*.   c. Ring of perithecia at the union of single ascospore cultures 2 and 12 after 35 days.

in the laboratory (Fig. 3, b). Cultures and sub-cultures of the caespitose peritheial type, on the other hand, produced only perithecia when grown under identical conditions (Fig. 3, a). This difference in behavior, observed between the isolates obtained from the two types, indicated that a study of single ascospore cultures of each might prove mycologically profitable.

Single ascospore isolates were obtained by making dilute suspensions of ascospores scraped from the tips of mature perithecia (Fig. 1, b). This ascospore suspension was then poured over 2 per cent plain agar plates. The excess water on the surface of the plate was decanted, leaving only a film over the surface holding the spores. Each plate was incubated at laboratory temperatures for 6 to 10 hours to permit the ascospores to initiate germ tubes. The plates were then placed beneath the microscope where the germinating ascospores were easily observed with the 10 × objective.

The single ascospore isolates were made by using a modification of the method described by Lambert (4). A wire loop was made by bending a piece of fine platinum wire around the tip end of a sewing needle, small enough to make the loop fit within the field of the 10 × objective when suspended beneath the objective. The wire loop was suspended in such a manner that it would hang immediately above the surface of the agar plate when the 10 × objective was in focus with an ascospore resting on the surface of the agar. When the "cookie cutter" was properly attached and adjusted, single ascospore isolates were made quickly and easily. The procedure consisted merely of locating a single, germinating ascospore on the surface of the poured plate and lowering the objective until the wire loop circumscribed a cut around the spore to be isolated. The plate was then transferred to a 30 × binocular and the small agar disc lifted out with a dissecting needle. The ascospore was not visible under the binocular but the circular cut made by the wire cookie cutter was clear.

Eighteen single ascospores from 42 trials with the caespitose perithecial type and 12 from 31 trials with the single perithecial type were obtained in pure culture. These were used in all subsequent studies involving single ascospore cultures.

CULTURE DEVELOPMENT OF SINGLE ASCOSPORE ISOLATES OF THE SINGLE AND CAESPITOSE PERITHECIAL TYPES

Successive sub-cultures of the 12 single ascospore isolates of the single perithecial type grown on potato-dextrose agar plates consistently produced nothing but pycnidia. The number of pycnidia produced by the different isolates was variable. In general, mature alpha spores were produced within 2 weeks after the plates were inoculated but spore production continued until the plates became too dry to sustain further growth.

When the 12 single ascospore isolates of the single perithecial type were cultured on steamed soybean stems, pycnidia were again produced by each isolate. The stems were cut into 4-inch sections and placed in test

tubes.   The bottom of each test tube contained moist filter paper to prevent rapid drying.   This permitted growing the cultures indefinitely if sterile water was added periodically.   Such cultures were carried for as long as 70 days but produced only pycnidia.   In general, the mature alpha spores were found within 10–14 days after the stems were inoculated and they were produced during the entire period the cultures were grown.   Beta spores were produced on sterile stems but they were never observed on agar media.

The 18 single ascospore cultures of the caespitose perithecial type were grown in the same manner and on the same media as were the 12 single ascospore cultures of the single perithecial type.   Each of the 18 cultures produced perithecia on agar media as well as on steamed stems.   From 5 to 42 perithecia have been observed arising from a single stroma on agar media.   When grown on sterile stems 1 to 7 perithecia, generally 2 to 4 occurred in caespitose groups.   Pycnidia have never been obtained in either mass or single ascospore cultures originating from the caespitose perithecial type.   The fact that it is homothallic is unquestionable.   Its failure to produce pycnidia, however, is somewhat confusing.

PRODUCTION OF PERITHECIA BY PAIRED SINGLE ASCOSPORE
CULTURES OF THE SINGLE PERITHECIAL TYPES

When repeated attempts to produce perithecia from single ascospore cultures of the single perithecial type failed, it was assumed that the organism might be heterothallic.   To test this assumption the 12 single ascospore cultures were paired in all possible combinations.   Pairings were accomplished by inoculating tubed, steamed soybean stems with agar-grown inoculum of the two cultures that were to be paired.   Two small pieces of agar, one of each of the two cultures being paired, were placed side by side on the steamed stems.   Since 12 single ascospore cultures were paired in all possible combinations, a total of 144 pairings resulted.   After all pairings were completed the cultures were incubated at laboratory temperatures and periodically checked for pycnidial and perithecial development.   Two sets of pairings were made and the results obtained were tabulated (Table 1).

The inoculated stems became covered with a white mycelial growth 3–5 days following inoculation.   Within 10–14 days pycnidia were generally produced 'over the entire surface of the stem.   Mature pycnidia always produced the alpha type conidia.   The beta type was usually produced simultaneously within the same pycnidial locule with the alpha type.   Production of the beta type, however, was irregular and attempts to associate its production with specific cultures or with a specific growth phase failed.

In the first experiment in which all 12 cultures were paired, the first perithecial beaks were observed after 35 days.   The beaks were produced singly and were long and black as shown in figure 3, c.   The beaks sometimes grew from the base of an old pycnidium or they developed independently and were not associated with pycnidial structures.   In general,

they tended to be produced in a collar around the stem between the two pieces of inoculum (Fig. 3, c). When produced at the base of a pycnidium, the perithecial cavity was imbedded in the base of the old pycnidial stroma, developing somewhat beneath and intermixed with the old, empty pycnidial cavities. When perithecia developed in the absence of pycnidial stromata, the beaks penetrated the black, dorsal, epidermal stroma and the perithecial cavities were imbedded in the cortical tissues of the stem, extending down to the vascular tissues. The occurrence of pycnidia and perithecia within the same lesion has never been observed except, as described above, where perithecia developed as a result of pairings.

The inoculated tubes were carried for 75 days before final readings were made and the tubes were discarded. The final reading showed that 33

TABLE 1.—*Perithecial production* by 12 single ascospore cultures of the single perithecial type when paired in all possible combinations in two separate experiments

Cul. No.	1	2	3	4	5	6	7	8	9	10	11	12
1	-	+*	*	-	+*	+*	+*	+*	+*	+*	+*	-
2	+*	-	-	*	-	-	-	-	-	-	-	+*
3	*	-	-	*	-	-	-	-	-	-	-	+*
4	-	*	+*	-	+*	*	+*	*	*	+*	+*	-
5	+*	-	-	+*	-	-	-	-	-	--	-	*
6	*	-	-	0	-	-	--	--	-	-	-	+*
7	+*	-	-	0	-	-	-	-	-	-	-	+*
8	+*	-	-	*	-	--	-	-	-	-	-	*
9	+*	-	-	0	-	-	-	-	-	-	-	+*
10	+*	-	-	*	-	-	-	-	-	-	-	*
11	*	-	-	+*	-	-	-	--	-	--	-	+*
12	-	+*	+*	-	+*	0	+*	*	*	+*	+*	-

a – Perithecia absent.
+ Perithecia present in first experiment.
* Perithecia present in second experiment.
0 Failures of expected pairings in both experiments.

of the 144 pairings had produced perithecia. The occurrence of these successful pairings are shown in table 1 as +.

Examination of this table shows that if cultures 1, 4 and 12 are accepted as opposites of cultures 2, 3, 5, 6, 7, 8, 9, 10, and 11, the expected successful combinations occurred in all cases except when 1 was paired with 3 and when 4 was paired with 2, 6, 8, and 9. Culture 3, however, did successfully pair with 4 and 12, and cultures 2, 6, 8, and 9 had paired with 1 and 12 in at least one trial.

Since this left 5 pairings which should have produced perithecia the experiment was repeated. In the repetition greater care was taken in setting up the tubes to prevent the inoculated stems from becoming dry. The experiment was carried for 75 days at laboratory temperatures. Final readings were made by examining each inoculated stem under a binocular. Whenever perithecial beaks were observed the observations were confirmed by mounting slides of ascospores under the microscope. The results obtained in this second attempt are shown as * in table 1. In the second

experiment a total of 50 pairings produced perithecia. The reasons why the other 4 pairings (6 × 4, 7 × 4, 9 × 4, and 12 × 6) were not successful are unknown. They were successful when made in the contrary direction. The successful combinations did, however, confirm the assumptions made from the data in the first experiment. Cultures 1, 4, and 12 reacted alike and 2, 3, 5, 6, 7, 8, 9, 10, and 11 reacted alike. The fact that perithecia were produced when any one culture of the 1, 4, and 12 group was combined with any one culture of the 2, 3, 5, 6, 7, 8, 9, 10, and 11 group was the basis for stating that the single perithecial type was heterothallic. Paired cultures on stem-meal agar produced only pycnidia.

<center>IDENTIFICATION OF THE TYPES</center>

As stated in the review of literature, the earlier treatments of the members of the genus *Diaporthe* were based largely on host relations; and it was not until the monograph of Wehmeyer (8) that morphological grounds replaced those of pathogenicity. At that time Wehmeyer combined those species with elongated ostiolar necks under the name *Diaporthe phaseolorum* and based the species on the form described by Harter (2) from Lima bean. With this form he set up the form causing pod and stem blight of soybean as var. *sojae* and that from sweet potato as var. *batatatis.* The first of these was described by Lehman (5), the second by Harter and Field (3). The latter variety was separated from the former by the fact that it had a valsoid stroma and smaller ascospores.

The heterothallic or single perithecial type of the Iowa material closely resembles the descriptions given by Lehman both in its gross appearance in the field and in culture and in the perithecium, ascus, and ascospore measurements. In both natural and cultural material the perithecia were borne singly on an effuse stroma, had long cylindrical beaks measuring from 347 to 521 μ in length in nature but often exceeding 2 mm. when grown in culture. The perithecia measured 156–260 × 192–335 μ. They were erumpent through a blackened zone on the surface of the stem with a black line often seen extending from the margin of the stroma into wood and running along the edge of the pith cavity. The asci were sessile, elongate, 8-spored, and measured 37.0–52.0 × 7.4–12.9 μ (Fig. 1, d). The ascospores were bicellular, slightly constricted at the septum and measured 10.4–18.5 × 3.7–5.5 μ (Fig. 1, e). Although the ascospore measurements were noticeably larger than the corresponding dimensions given by Lehman, this difference is not believed to be significant; and the authors are inclined to follow Wehmeyer and consider the heterothallic form as *Diaporthe phaseolorum* var. *sojae.* The presence of heterothallism would explain the variations among strains of "perithecial and conidial" forms reported by other workers. Heterothallism hitherto has not been reported for the genus.[2]

[2] Cayley (1) suggested that heterothallism was present in *Diaporthe perniciosa* Marchal but her statement concerned the irregular occurrence of the conidial phases and was not connected directly with the appearance of the perithecial stage and so was not used in the present accepted sense of this word. Wehmeyer (8) also pointed out that Cayley was not dealing with our present concept.

The identity of the homothallic caespitose form is somewhat more difficult to establish. It was distinct from *D. phaseolorum* var. *sojae* in that it actively attacked the stems of the soybean; large light to dark brown lesions circumscribed the stems and the plants wilted and died. Pycnidia have never been found associated with these lesions nor have they appeared in any of the cultures. Single ascospore isolates repeatedly produced perithecia in culture. Perithecia occurred naturally also on overwintered stems in caespitose groups of 1 to 7 per stroma, predominantly in groups of 2 to 4. These perithecia measured 178–325 × 275–390 μ, being more globose (not flattened) than *D. phaseolorum*. In culture the stromata were limited (Fig. 2, b) to circular areas on the surface of the plate. On stem cultures the black line did not extend into the stelar region but was confined to the surface through which the beaks of the perithecia emerged. The beaks were 280 to 546 μ in length and slightly more tapering than the beaks of *D. phaseolorum* (Fig. 1 and 2). The asci were elongate, sessile, with thin evanescent walls and measured 27.2–40.8 × 6.8–8.5 μ. The ascospores were bicellular and measured 8.5–10.2 × 3.4–5.1 μ (Fig. 2, c). The caespitose habit, the globose perithecia, the tapering beaks, the smaller ascospores and the homothallism make it impossible to consider the caespitose type the same organism as described by Harter on Lima bean or by Lehman on soybean. It resembles most closely the strain of *D. phaseolorum* described by Tucker (7) except that it does not produce conidial spores, neither alpha nor beta, such as he figures in his strain on pepper. In this latter respect, it is close to the strains of *D. arctii* reported from Europe on *Melilotus* and *Medicago* (8, p. 7). However, if we accept Wehmeyer's separation on the length of ostiolar beaks, the caespitose organism could not be that species. The authors, therefore, consider it to be a perithecial strain of *Diaporthe phaseolorum* var. *batatatis*, differing from the strains previously described by its lack of a conidial phase, and also in its pathogenicity.

Preliminary infection studies with isolates obtained from both perithecial types have shown that either can infect the soybean stem. Cultures of the caespitose type inoculated into plants growing in the greenhouse have produced the typical symptoms observed in natural infections in the field; lesions circumscribing the stem and causing the plants to wilt and die prematurely. Cultures of the single perithecial type produced infections only on maturing stems. Isolates of the caespitose type are, therefore, considered to be much more pathogenic than isolates of the single perithecial type.

SUMMARY

In the course of investigations of soybean diseases, two members of the genus *Diaporthe* were found which were different relative to pathogenicity and type of perithecial development. One was heterothallic with scattered single perithecia and the other was homothallic with caespitose clusters of perithecia. The former produced typical Phomopsis conidia of the alpha

and beta types; the latter lacked conidial stages. The heterothallic form was recognized as *Diaporthe phaseolorum* var. *sojae;* the homothallic as *Diaporthe phaseolorum* var. *batatatis.* The latter variety actively attacked soybean stems, girdling them and causing the plants to wilt and die. The former variety was less pathogenic, attacking mainly mature plants and producing linear rows of pycnidia on branches and stems.

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AND

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2,4–D INJURY TO COTTON FROM AIRPLANE DUSTING OF RICE[1]

A. A. DUNLAP

During the summer of 1947, considerable damage occurred to cotton in the Gulf Coast area of Texas where nearby rice fields had been dusted with 2,4-D[2] by airplane. Injury to cotton from this source was reported from several coastal counties between Corpus Christi and Beaumont. The greatest damage probably occurred in the Bay City area. A committee of rice and cotton growers found varying degrees of injury on about 10,000 acres of cotton in Matagorda and Wharton counties and they estimated a total loss amounting to 2000 acres. Based on an average yield of one-half bale per acre, losses in both lint and cotton seed were estimated at around $200,000 for these two counties alone.

A warning of the danger to cotton from minute quantities of 2,4-D was sounded by Staten (7, 8) as early as June, 1945. Later, Johnson (5) reported the extreme sensitivity of certain plants to the vapors of 2,4-D that were given off by uncleaned spray equipment. In California, damage to grapevines from 2,4-D was noted by Harvey and Robbins (4) as possibly due to drifting of herbicidal spray. In regard to cotton injury from rice dusting, Brown (2) observed damage in Louisiana, in 1947, and preliminary note has been made (3) of the observations herein reported from Texas. The carrying-over of 2,4-D damage into the seed was noted by Pridham (6) in the case of red kidney bean plants which were sprayed with 2,4-D while the pods were ripening. He found that the seedling offspring showed typical 2,4-D symptoms.

In the areas where these observations were made, rice fields were dusted with 2,4-D during the latter part of May and the first week in June. The cotton had been planted on various dates during March and April. The first injury was noted on the cotton about the tenth of June, at which time the rice growers in most areas discontinued dusting. Regular visits were made throughout the growing season to certain selected fields in order to obtain information as to persistence of the effects of the chemical on the plant and seed. Owing to the variation between fields in regard to such factors as age of plants, fertility, rainfall, and care of the crop, no attempt was made to obtain yield data from damaged fields for comparison with fields that were not affected by the 2,4-D dust.

In some cases, cotton fields showing 2,4-D injury were found at estimated distances of from 15 to 20 miles from the nearest rice fields. However, airplanes with leaky dust-containers may have passed much closer

than this to some of these fields during or following the dusting of the rice. The fact that cotton fields of 20 acres or more usually showed fairly uniform damage over the entire field would indicate that the dust had drifted for a considerable distance. According to a table by Brooks (1), water droplets about 5 μ in diameter can drift over three miles in falling ten feet when the velocity of the wind is 3 miles per hour. The particle size of the dust used on the rice in this area varied from 4 to 16 μ and the airplanes probably attained heights of from 100 to 200 feet while turning. With wind velocities possibly as high as 10 miles per hour, some of the dust could have drifted, therefore, even farther than the distances mentioned. Since the commercial applications by the airplanes consisted of about 10 lb. of a

FIG. 1. A. Cotton plants with distorted foliage in a relatively dry field two months after 2,4-D symptoms were first evident. Plants that had set some bolls prior to 2,4-D contact often showed poor recovery even in fields with favorable soil moisture. B. Abnormal cotton squares (flower buds) caused by 2,4-D; normal square at left.

10 per cent dust per acre, the actual 2,4-D dosage on the cotton must have been extremely light in most instances, especially where the cotton was a mile or more from the rice. It is also of interest that one of the weeds (*Caperonia palustris*), for control of which the rice fields were dusted, was often found apparently unharmed among badly injured cotton plants. Occasionally, fields with no 2,4-D symptoms were found in the same vicinity with damaged fields. Protection was apparently provided in some instances by rows of trees on the windward side of the field.

Apparently the cotton plant is extremely sensitive to slight traces of 2,4-D. In spite of such high susceptibility, the plants showed remarkable recovery from severe 2,4-D symptoms. Most of the cotton in this area consisted of Stoneville and Deltapine strains; the latter variety sometimes appeared to be affected less severely than the former by this chemical.

Symptoms of 2,4-D damage, as found in these fields, are shown in figures 1 and 2. The injury consisted entirely in abnormal growth of the foliage, stems, and fruiting structures. No cotton plants were found that were known to be killed by the 2,4-D. The distorted leaves with scalloped margins, narrow lamina, and long tentacle-like teeth persisted on the plants for several weeks, until the middle of August or later. The cotton stems in badly damaged fields were often swollen and the cortex broken open (Fig. 4, A). Frequently spherical, gall-like stem swellings occurred near the ground. Flower buds or squares (Fig. 1, B) and bolls were also ex-

FIG. 2. Cotton leaves showing 2,4-D injury; three injured leaves on left and a normal leaf of the same age on right.

ceedingly malformed. Usually these abnormal fruiting structures were eventually shed from the plant but in some cases elongated or one-sided bolls were produced which frequently had only one or two locules instead of the normal four or five "locks." Although small squares sometimes dried up on recently affected plants, there was no tendency towards excessive shedding of the large squares and young bolls in the fields under observation.

The extent of the original injury seemed to depend to a large degree on the 2,4-D dosage and possibly also on the rate of growth of the plant. The mildest symptoms consisted of a few slightly distorted leaves near the top of the plant. In case of severe injury, all of the new growth was ab-

normal (Fig. 1). Crop losses varied from no apparent loss in slightly af-
fected fields to complete loss in others. Recovery seemed to depend on
maturity of the plants, soil moisture, and soil fertility conditions. In
fields that received favorable rainfall during the summer, the 2,4-D in-
jured plants made good recovery in most cases. New growth occurred
often through the development of lateral branches (Fig. 3) and the origi-
nal central stalk usually ceased to grow. In some fields, the main stems
increased in height and nearly normal bolls were formed among the dis-

FIG. 3. Cotton plant showing recovery from serious early damage from 2,4-D (dead
portion of main stem against white background). Normal bolls were formed on strong
lateral branches that developed later.

torted leaves which continued to appear for a month or two after the first
injury. In most cases, however, especially in plants that were relatively
young (prior to first bloom) at time of dusting, strong lateral branches
were developed from the lower nodes on the stem and these vegetative
branches produced apparently normal leaves, squares, and bolls.

Seed collected from plants that had recovered from initial injury ger-
minated only 40 to 60 per cent. Also, emergence from non-sterile soil
was slower with these seed than with seed from normal plants harvested at
the same time. In the case of seed from bolls that developed among in-
jured foliage, the tips of the hypocotyls after emergence from the seed
coat showed swellings close to the root tip, as shown in figure 4, B. It

seemed remarkable that about 50 per cent of the seedlings from these seed, which were picked in September from bolls that were formed several weeks after the original damage occurred, should show this 2,4-D symptom. Another lot of these seed that was germinated four months later (January, 1948) still showed the same abnormal root development upon sprouting. The cotyledons were not observed to be noticeably distorted by the 2,4-D. The first (and sometimes the second) true leaf was often deformed or filiform in shape and the seedlings were slower than the normal ones in

FIG. 4. A. Swelling of cotton stem and cracking of bark due to 2,4-D injury. B. Cotton seedlings with swollen root tips from seed produced by 2,4-D-affected plants. Such seedlings were delayed in emergence and early growth was abnormal. Two normal seedlings are shown on the left.

their early growth. Seedlings from plants experimentally sprayed with 2,4-D required a month or more for emergence, apparently because of inhibition of root development. They outgrew the injury eventually, however, and made normal growth after producing a few distorted leaves.

Continued care and cultivation of cotton fields in the usual manner would seem to be the best procedure to follow in case of 2,4-D damage. This would be particularly advisable in cases of slight damage or where the plants had not reached the flowering stage at time of 2,4-D contact. Retarding of growth was one of the most serious consequences of the 2,4-D injury, especially in the case of relatively mature plants that had a crop

of bolls set when the injury occurred. Continued dry weather further retarded the growth of cotton in some fields. Boll weevils and boll worms become more numerous in most cotton growing areas as the season advances. For this reason, the delayed fruiting of cotton plants that have been damaged by 2,4-D may produce a light crop due to increased insect damage to the new squares and bolls. Therefore, more-than-usual attention to insect control is necessary. Owing to the danger of poor germination and seedling injury, seed from cotton plants that have been affected with 2,4-D should not be saved for planting purposes.

Prevention of damage to susceptible plants or crops growing in nearby fields would necessitate application of 2,4-D weed killers by spraying at low pressures (less than 100 lb. per sq. in.) with nozzles that do not produce a mist. It would also appear best to make the application when the wind velocity is not greater than 3 to 5 miles per hour. Application of 2,4-D by airplane should be made at as low an altitude as possible and care should be taken to avoid high-altitude turning or traveling over susceptible crops in passing to and from the loading point. All airplane spraying equipment should be provided with positive shut-off devices near the nozzle to prevent dripping or leakage. Relatively non-volatile forms of 2,4-D, such as the sodium or triethanolamine salts, should be used whenever possible. Until more is known concerning the dangers and limitations in the use of 2,4-D for killing weeds, extreme carefulness and caution should be maintained at all times. Further costly error in the premature and improper use of the chemical may result in unfortunate delay in the development of these highly-useful hormone-type weed killers.

SUMMARY

1. Symptoms of 2,4-D injury on cotton, extent of damage and recovery, and effects on cotton seed were observed in Texas Gulf Coast fields in 1947, following dusting of rice fields by airplane.

2. Partial recovery of cotton plants from 2,4-D injury, as shown by the production of normal foliage and fruiting structures, occurred in most fields where favorable conditions for growth prevailed during the remainder of the season.

3. In some cases, seed from plants affected by 2,4-D showed poor germination and produced seedlings with swollen root tips (hypocotyls) together with other abnormalities.

4. Possible steps are suggested for the prevention of 2,4-D damage to susceptible crops in nearby fields.

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THE EFFECTS OF MEALYBUGS FEEDING ON PINEAPPLE PLANTS GROWN IN FINELY ATOMIZED NUTRIENT SOLUTIONS[1]

WALTER CARTER

This project was initiated in order to determine the sequence of symptoms of mealybug wilt. It was known that when plants showed wilt symptoms in the field, the roots were collapsed, but whether this root collapse was primary could not be determined under field conditions with any degree of certainty. A test to study the symptom sequence on pineapple plants grown in both sand and water culture met with similar difficulty. It was not until the method of growing the plants in finely atomized nutrient solutions (2) was devised that it became possible to follow with a considerable degree of accurate measurement, the whole symptom sequence following the infestation of plants with mealybugs.

PROCEDURE

Plants were grown in what has been described as a water-vapor box. This is a simple wooden cabinet with holes drilled in the top into which planting material pieces are fit. Nutrition is provided by means of a finely atomized spray of nutrient solution which circulates continually around the base of the plant. Growth, both of roots and aerial portions of the plant, is very rapid under these conditions, and both roots and leaves can be examined with ease at any time (Fig. 1).

After two pilot runs to establish the technique, the first detailed experiment was set out. Slips from a section of a field free of mealybugs were cured and set in three cabinets holding eighteen plants each. Planting pieces were weighed prior to setting and were distributed in such a manner that each box had a uniform slip weight distribution. Three weeks after setting, measurements of five roots of each plant were made, and these were continued each week until the end of the experiment. Six weeks after the plants were set in the cabinets, they were infested by the fruit skin method (4), two of the cabinets being used for infested plants and one as an uninfested check. It should be recalled that this method of infestation usually involves large numbers of mealybugs in all stages. All the infestations were sprayed out after ten days.

EXPERIMENTAL RESULTS

The Sequence of Symptoms

The sequence of symptoms, of primary interest in the first experiment, is shown in table 1. Root symptoms were rather clearly defined, first by
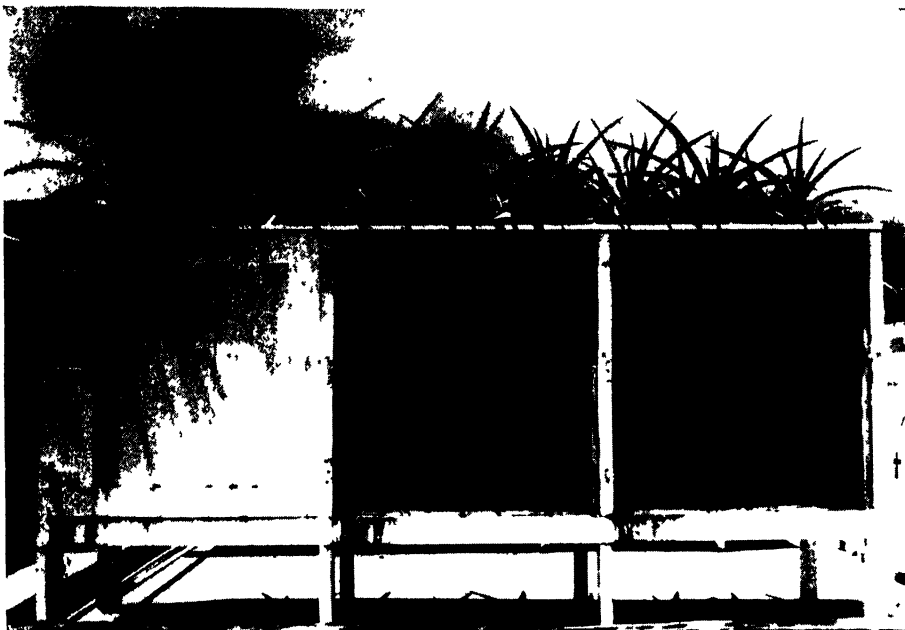
FIG. 1. A general view of the water vapor cabinet. Two doors were removed to show the root growth.

cessation of elongation and second by a soft collapse of the entire root; the second phase follows closely on the first. Some plants develop leaf symp-

TABLE 1.—*Sequence of symptoms from first indication of cessation of root elongation to appearance of leaf symptoms following infestation with mealybugs*

Plant No.	No. of days after mealybug infestation		
	Root elongation affected	First dead roots	First leaf symptoms
3	42	49	63
3	42	49	63
2A1	42	52	66
4	42	49	54
2	42	58	63
5	49	58	63
3	49	58	63
6	49	49	54
3	49	58	63
4	49	58	63
6	49	49	63
2C2	49	58	63
3	49	49	63
5	56	61	73
1C1	56	58	63
2	56	58	70
2C6	56	76	76
1B1	62	76	70[a]
2B1	62	76	76
2	69	76	76
1A1	72	79	82

[a] 1 median leaf only affected.

toms without the loss of all the roots.   The development of new roots is variable, sometimes occurring during the period when the old roots are collapsing, sometimes only after a considerable delay.   Leaf symptoms are typical but a very rapid recovery usually occurs which prevents the development of extreme third and fourth stages as encountered in the field (Figs. 2 and 3).
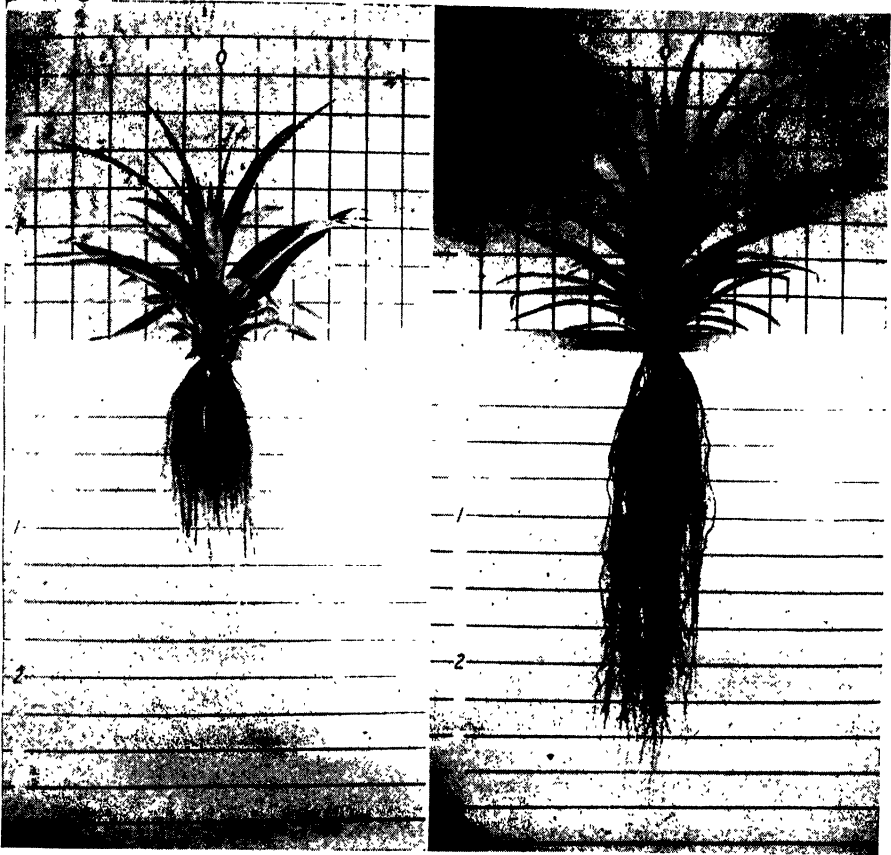
Fig. 2.   (Left) Wilted plant with center recovering.   Note mass of collapsed roots with short new white roots emerging.
FIG. 3.   (Right) Normal uninfested plant.

Table 2 gives some details of root elongation measurements.   It should be borne in mind in connection with this table that if a measured root ceased elongating and collapsed, a live root was substituted so that as long as there were live roots on the plant, elongation measurements were continued.   The collapse of a root system is progressive.   All the roots do not die simultaneously, although once collapse has started, it proceeds rapidly. New roots emerging after the original root mass has died, or during the period of its collapse, soon attain the elongation rate of roots on normal plants.

FIG. 4.

ROOT ELONGATION IN cms.

WEEKS ELAPSING AFTER INFESTATION

PLANT WEIGHT INCREASE 77 PER CENT

FIG. 5.

ROOT ELONGATION IN cms.

WEEKS ELAPSING AFTER INFESTATION

PLANT WEIGHT INCREASE 116 PER CENT

FIG. 6.

ROOT ELONGATION IN cms.

WEEKS ELAPSING AFTER EXPERIMENT STARTED

PLANT WEIGHT INCREASE 142 PER CENT
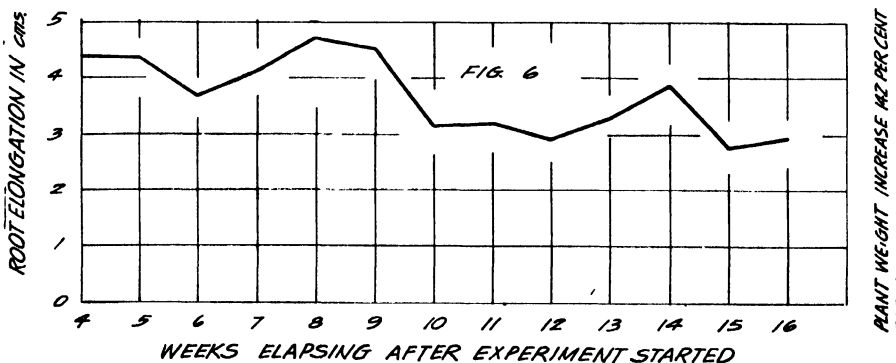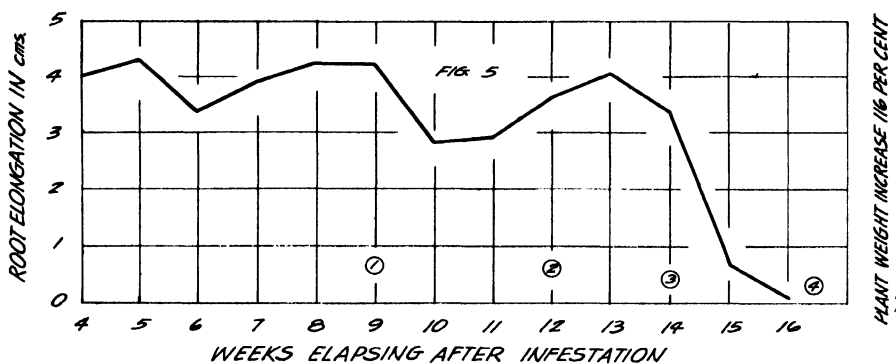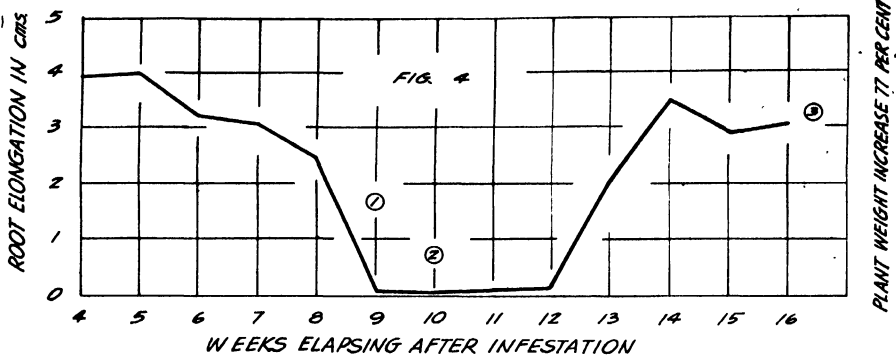
FIG. 4. Plant 1B1 showing typical root collapse with development of new roots.
1. Long roots dead, median leaves reddening on one side.
2. Long roots dead, several white tipped new roots, median leaves reddish.
3. Median leaves wilted, center growing.

FIG. 5. Plant 1A4 showing a case of delayed root collapse.
1. Leaves and roots normal.
2. One median leaf yellow, roots normal.
3. Roots normal.
4. One median leaf yellow distal of feeding area, median whorl with tips wilted. Old roots dead.

FIG. 6. Mean of 18 uninfested check plants.

Table 3 summarizes all the measurements with respect to final symptom class, which for this experiment were as follows; 0 = healthy uninfested plants showing no symptoms, 1 = infested plants showing no root or leaf symptoms, 2 = typical wilt symptoms on plants showing recovery, and 3 = a single case of advanced third stage symptoms. For the infested but symptom-free plants, there is a reduced percentage weight increase and what is probably a significantly reduced length and width of the leaves, with no apparently significant difference in the root number. This was

TABLE 2.—*Root elongation. Summary of weekly means for each plant which showed typical wilt symptoms*

Symptom class[a]	Elongation (in cm.) for week following mealybug infestation							
	4th	5th	6th	7th	8th	9th	10th	11th
3	3.36	2.72	2.74	1.54	0.50	0.06	0.24	0.00
2	3.80	4.28	0.82	0.04	0.04	0.00	3.34	3.88
	3.46	3.12	1.20	0.30	0.16	0.60	1.80	2.12
	4.42	3.34	1.26	1.08	0.94	1.14	1.96	2.56
	3.46	4.64	1.86	0.10	1.36	1.08	1.78	2.74
	4.04	3.90	1.88	0.38	0.14	0.00	2.02	1.62
	3.54	3.98	2.22	0.08	0.10	0.20	1.60	2.18
	4.12	4.62	2.44	0.30	0.28	0.54	2.06	2.36
	4.22	4.68	2.84	0.36	0.68	1.02	2.10	3.46
	4.42	3.66	2.20	0.60	0.66	1.42	3.41	3.90
	4.74	4.58	3.96	0.76	0.10	0.30	0.04	0.00
	3.80	4.16	3.42	1.14	0.08	2.88	1.90	2.40
	2.64	4.90	4.74	3.60	0.60	0.00	0.06	0.72
	4.90	4.54	4.62	3.28	0.80	0.10	0.24	1.94
	3.46	3.54	2.80	2.80	0.98	0.36	0.02	1.70
	3.68	3.98	3.86	3.44	1.72	0.28	0.00	1.54
	4.06	3.30	3.40	4.00	2.84	0.78	0.08	0.00
	4.00	3.94	3.36	2.78	2.72	0.98	0.22	0.08
	4.88	5.24	4.84	4.06	4.40	3.20	0.44	0.48
	4.36	4.44	2.64	3.76	3.86	1.18	0.10	0.10
1[b]	3.93	4.29	3.75	3.85	3.96	3.60	2.88	3.25
0[c]	4.34	4.60	4.15	4.09	4.05	3.73	4.01	3.68

[a] 0 = Healthy uninfested plants showing no symptoms.
 1 = Infested plants showing no root or leaf symptoms.
 2 = Typical wilt symptoms on plants showing recovery.
 3 = A single case of advanced third stage symptoms.
[b] Means of 15 infested but symptom-free plants.
[c] Means of 18 uninfested check plants.

the first indication that a short infestation of mealybugs, insufficient to cause wilt on a particular plant, measurably affected the plant's ability to grow. The increased number of roots in class 2 reflects the development of the flush of new roots with which recovery is associated.

Infestation of Clonal Material in Vapor Boxes and in the Field

In the second experiment, varying numbers of mealybugs were used. Ten-, 50-, and 250-bug lots were applied to each of 12 plants while 18 plants were kept as uninfested checks. On the same date that these plants were infested, a similar series of plants growing in the field were infested

TABLE 3.—*Summary according to symptom class at time of harvesting the plants*

Data recorded	Symptom class[a]			
	0	1	2	3
Number of plants	18	15	20	1
Slip weight (gm.)	208	206	210	194
Final plant weight (gm.)	563	474	386	300
Increase in plant weight (per cent)	170	130	87	55
Final number of roots	61	63	76	61
Total length of live roots (cm.)	2696	2488	475	0
Total length of dead roots (cm.)	24	14	1395	1521
Dry weight of root mass (gm.)	10.8	10.6	5.8	5.1
Mean length of 10 longest leaves (cm.)	49	42	38	36
Mean length of 10 widest leaves (cm.)	4.4	3.9	3.6	3.5
Number of green growing leaves	20	20	19	21
Total number of leaves	55	54	49	42

[a] 0 = Healthy uninfested plants showing no symptoms.
1 = Infested plants showing no root or leaf symptoms.
2 = Typical wilt symptoms on plants showing recovery.
3 = Advanced 3rd stage wilt.

at the same time with mealybugs from the same lot. All infested plants were sprayed out eight and ten days later. Slips were used as planting material and all the slips in both the water vapor boxes and the field plots came from the same clone. In addition to this, plants used in the water vapor boxes were so distributed that slips from individual mother plants were distributed as evenly as possible throughout three water vapor boxes. When the field infestations were made, the series were separated by removing plants to provide a space between each set of infested plants. The numbers of roots on these plants were counted to provide a sample with which the water vapor box plants could be compared with respect to numbers of roots. These data are in table 4, and it will be noticed that the water vapor box plants had a very much larger number of roots than did the field plants.

Only six plants growing in the boxes showed wilt symptoms and in these, five separate mother plants were represented. Five of the plants wilting had been infested with 250 mealybugs each; one with 50 mealybugs.

TABLE 4.—*Experiment 2: Number of roots when plants were infested compared with those on a sample number of field plants*

Number of roots							
Field	Box 1	Box 2	Box 3	Field	Box 1	Box 2	Box 3
3	42	52	71	22	32	53	44
21	73	46	107	4	25	41	73
13	44	67	55	46	66	49	60
32	41	43	62		55	66	46
9	60	55	59		91	94	91
21	25	64	66		77	42	48
6	54	69	56		50	42	37
16	68	54	48		59	52	79
18	87	59	48		63	71	64

Only two of the field plants infested with 250 mealybugs wilted and none following the smaller infestations. This difference between susceptibility in soil and soilless conditions for growth is worthy of note although it has not been consistent throughout these experiments. Soil complexes as effecting susceptibility to wilt have been the writer's concern in previous studies (1, 3, 4). This datum suggests that a factor sometimes present in soils is negative for susceptibility.

The symptom sequences for two of the plants wilted in the water vapor box are shown in figures 4 to 6.

Figure 4 shows the typical symptom sequence in relation to root elongation. There is first a drop in the elongation rate and roots begin to die. All the roots collapse and the typical leaf wilt begins. New roots emerge and the center of the plant resumes growth.

Figure 5 shows a case of delayed interference with root elongation. In this case, one median leaf turned color distal of a heavy concentration of mealybug feeding spots which lay in an almost continuous band about 1 inch wide across the leaf, one month before root failure was apparent. The final wilt symptoms in this case were very mild, extending only to the tips of a median whorl of leaves when the experiment was concluded.

Figure 6 is for the means of the uninfested check plants.

Infestation of Clonal Material for Varying Lengths of Time

The third experiment duplicated experiment 2 with regard to the use of a single clone and the distribution of sister planting pieces from the same mother plant. The number of bugs used was varied however, as well as the length of time they were permitted to feed before the plants were sprayed. Numbers of bugs used were 20, 100, and 500. One set of plants was sprayed out after one week and the second after two weeks. Infestations of field plants were made at the same time with mealybugs from the same sources, and these were sprayed out at the end of the first week. Counts were made of a sample of field plants as described in Experiment 2, with the same result as in that experiment, that is, that the roots of the water vapor boxes were very much greater in number than in the field plants. The experiment was terminated 3 months and one week after the infestations were made.

The use of as many as 500 mealybugs to a plant in a situation open to predators and protected from ants resulted in a general attack by predators so that actually by the end of the second week, the colonies in the unsprayed lot were already greatly reduced. Two weeks after infestation and one week after the first set of plants had been sprayed, an estimate was made of the number of mealybugs on each plant before the second lot were sprayed out. These figures represent a minimum number of bugs on the plant at the time because it is impossible to count all the mealybugs on a pineapple plant unless the plant is dissected. Three sprayed plants originally infested with 500 bugs each, had 1 to 3 bugs visible. The un-

TABLE 5.—*Comparison of wilt incidence in plants grown in field plots and in water vapor boxes.   Experiment 3*

No. of mealybugs	Field		Water vapor	
	No. of plants	No. plants wilted	No. of plants	No. plants wilted
20	12	1[a]	12	0
100	12	5	12	1
500	6	5	12	8

[a] 1 median leaf wilted at tip only.

sprayed plants showed colonies of from 15 to 40 in the 500-bug lot, 9 to 23 in the 100-bug lot, and 1 to 2 in the 20-bug lot with 2 out of 6 showing no bugs.   Actually there was no difference in the number of plants wilting following the two feeding periods.   In the field where infestations were made in an ant occupied area, wilt incidence was greater than in the boxes, as shown in table 5.

In view of the large number of mealybugs used in this test, the reaction of individual plants is of interest.   Table 6 permits a comparison between sister plants within the single large clone from which all the plants used were derived.   There was no effect on percentage weight increase of plant that could be ascribed to the insect's feeding when wilt did not occur. The susceptibility of sister plants differed because among nine plants wilted, 7 individual mother plants were represented.   Further comparisons are

TABLE 6.—*Comparison of weights, in grams, of plants derived from slips of same mother plants. All the mother plants belonged to one large vegetative clone.   Experiment 3*

Mother plant No.	Box 4–infested			Box 5–infested			Box 6–check		
	Slip 1			Slip 2			Slip 3		
	Initial slip wt.	Final plant wt.	Inc. (Pct.)	Initial slip wt.	Final plant wt.	Inc. (Pct.)	Initial slip wt.	Final plant wt.	Inc. (Pct.)
A1	320	860	169	380	720	90[a]	340	840	147
A2	420	750	79[a]	440	1340	205	420	1340	219
A3	420	850	102[a]	400	1040	160	380	1040	174
A4	480	810	69[a]	460	1020	122[a]	420	1070	155
A5	420	1200	186	390	800	105[a]	430	1270	195
A6	400	980	145[a]	380	930	145[a]	390	1100	182
B1	540	1400	159	500	1280	156	570	1430	151
B2	410	1260	207	400	1330	233	440	1280	191
B3	410	1190	190	400	1110 ·	178	400	960	140
B4	380	1020	168	400	1050	163	400	1120	180
B5	560	1570	180	520	1460	181	500	1380	176
B6	380	1080	184	370	840	127[a]	380	1100	190
C1	470	950	102	430	1060	147	420	1100	162
C2	400	1130	183	350	1050	200	360	1120	211
C3	380	940	147	370	1040	181	380	1200	216
C4	530	1030	94	530	1190	125	570	1270	123
C5	450	1150	156	440	1310	198	450	1300	189
C6	450	1200	167	420	1250	198	450	1390	209

· Plants showing symptoms.

available from table 7, in which the root elongation data and the detailed symptom record are listed. Plants 4A4 and 5A4 were sister plants. One had developed root symptoms early and these were followed by severe wilting. The other did not develop root symptoms until three weeks later and leaf symptoms at the conclusion of the experiment were mild. The

TABLE 7.—*Comparison of root elongation of wilted plants with that of symptom-free plants. Experiment 3*

Plant No.	Mealy-bugs used	Elongation (in cm.) for week following mealybug infestation									Notes on symptoms
		4th	5th	6th	7th	8th	9th	10th	11th	12th	
4A2	500	3.12	4.40	2.02	0.28	0.12	0.16	0.28	0.20	0.54	Typical severe wilt, 4th stage on median leaves. Center growing.
4A3	500	3.40	4.82	4.32	1.14	0.04	0.12	0.02	0.00	0.00	Do, but not so severely wilted.
4A4	500	2.94	3.70	2.66	1.72	0.18	0.14	0.06	0.30	0.00	As for plant 4A2.
4A6	500	2.92	3.60	4.82	3.30	4.10	2.60	1.70	0.40	0.18	Second stage wilt on one leaf, first stage on others.
5A1	500	3.24	4.88	1.00	0.18	0.16	0.26	0.20	0.28	1.44	Typical third stage wilt. Center growing.
5A4	500	3.58	5.20	4.68	3.30	3.72	2.80	1.54	0.62	0.00	Second stage wilt on two median leaves, first stage on others.
5A5	500	3.34	3.92	4.48	2.60	1.10	0.32	0.08	0.20	0.00	Typical second stage wilt on median leaves.
5A6	500	2.86	4.18	4.08	3.42	3.96	1.62	1.10	0.20	0.00	Second stage wilt on two median leaves, other leaves slightly offcolor.
5B6	100	3.76	4.62	4.80	2.02	0.42	0.06	0.04	0.00	0.00	Typical third stage wilt. Center growing.
4C3	20	4.10	4.48	4.54	3.56	4.32	3.16	2.48	1.56	0.00	No leaf symptoms developed by end of 12th week.
Mn.[a]	500	3.17	4.12	4.34	3.57	3.61	2.59	3.23	2.88	4.73	
Mn.[a]	100	3.37	4.07	4.02	3.00	3.59	2.61	3.01	2.76	3.79	
Mn.[a]	20	3.16	4.04	3.90	3.22	3.50	3.33	3.11	2.70	3.49	
Mn.[b]	0	3.09	4.00	4.01	3.12	3.52	2.59	2.86	2.47	3.17	

[a] Means of symptom-free plants.
[b] Means of check plants.

other pair of sister plants which wilted were 4A6 and 5A6. In both these cases, root symptoms were slow in developing and leaf symptoms were mild. Among the 8 wilted plants infested with 500 mealybugs there is considerable variation in time of onset of root symptoms but a fair agreement between early effect on roots and severity of leaf symptoms. Evidence of root elongation symptoms among the plants infested with 500 bugs, which nevertheless showed no leaf symptoms, is slight; only one of

the four plants in this category, (4A5), showed any root elongation reduction and that for one week only.

Restricted Feeding of Mealybug Strains

In experiment 4 the two clearly defined strains of mealybugs, green spotting and non-green spotting, were compared, using two methods of infestation. In one case the mealybugs were caged on a median leaf as far down as possible. Actually it is not feasible to affix the cages as low down as the insects normally feed. In the second case the mealybugs were applied free to the center of the plant and allowed to settle and move at will.

From 100 to 200 mealybugs of each strain were used in each case. When 200 bugs were caged on a single plant, 2 cages were used, one to each of two leaves, each cage containing 100 mealybugs.

Field plants were also infested at the same time. A comparison of the

TABLE 8.—*Comparison of wilt incidence in plants grown in field plots and in water vapor boxes. Experiment 4*

No. of mealy-bugs	Strain[a]	Location	Field		Water vapor box	
			No. plants	No. wilted	No. plants	No. wilted
100	G.S.	Free	12	7	6	2
100	N.G.S.	Free	10	3	6	3
100	G.S.	Caged	8	3	8	3
100	N.G.S.	Caged	8	0	10	0
200	G.S.	Free	6	5	6	4
200	N.G.S.	Free	6	0	6	4
200	G.S.	Caged	10	3	10	3
200	N.G.S.	Caged	10	0	8'	1

[a] G.S. = Green spotting strain
N.G.S. = Non-green spotting strain.

relative wilt in the two types of planting is shown in table 8. Although the numbers of plants in each class are too small for final conclusion, it is indicated that free living mealybugs, able to move around the typical area of feeding, are more effective than caged bugs in inducing wilt. In the field the green spotting mealybugs were more effective than the non-green spotting strain.

Effect on root elongation and root collapse was typical for both strains of mealybugs.

Continuous and Intermittent Infestations

In experiment 5 the effect of small colonies of mealybugs was tested, with the manner in which they were applied being the only variable. One set of 18 plants was infested with 10 mealybugs to each plant, three times, at approximately monthly intervals, the second and third infestations being added to the few bugs remaining from the previous colonies. The second set was infested similarly but each infestation was sprayed out after two weeks. There were therefore two weeks' freedom from mealybug feed-

TABLE 9.—Root growth data from plants infested 3 times with 10 mealybugs. Experiment 5

| Plant No. | Symptom class[c] | Root elongation (in cm.) for week following first mealybug infestation | | | | | | | | | | Root number and length | | | | |
		10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	Total No.	No. alive	Length (cm.)	No. dead	Length (cm.)
4A4	2	2.72	2.08	0.24	0.00	0.72	0.50	0.00	0.00	0.00	0.00	76	0	0.0	76	46.8
4C6	2	2.04	2.32	0.34	0.10	0.32	0.12	0.08	0.64	1.10	1.10	65	17	83.9	48	53.8
4A2	2	3.02	4.28	2.66	0.34	0.00	0.10	0.00	0.00	0.00	0.00	69	0	0.0	69	46.8
4C1	2	1.12	2.26	1.60	1.80	0.16	0.54	0.00	0.00	0.38	0.20	61	10	45.5	51	53.1
Mean[a]	0	2.74	3.55	3.12	3.30	2.92	3.05	2.86	2.96	2.44	2.60	65	62	72.2	3	27.0
5A4	2	2.06	1.84	2.30	2.38	2.08	1.04	0.16	0.00	0.08	0.00	89	15	35.6	74	54.8
5A2	1	0.20	1.26	1.18	0.90	1.12	1.54	1.06	0.84	0.62	0.12	55	29	42.6	26	45.2
5B3	1	0.32	0.62	0.76	0.24	0.70	1.26	1.18	1.04	0.20	0.00	60	41	52.3	19	55.7
5B5	1	1.22	0.94	1.04	1.08	1.46	1.84	1.32	1.28	0.00	0.08	47	41	45.8	6	28.9
5C6	1	1.56	1.96	0.76	1.00	1.50	0.50	1.06	1.54	0.52	0.24	49	43	57.7	6	54.9
5C3	1	1.12	1.28	0.98	0.32	1.16	1.86	1.60	2.16	0.70	0.16	54	41	51.3	13	30.3
5A6	1	1.38	1.16	1.92	2.10	2.30	2.70	2.14	2.80	1.24	1.36	87	79	47.3	8	26.8
Mean[a]	0	1.07	0.93	1.22	1.04	1.14	1.49	1.11	1.78	1.36	1.19	65	54	50.7	11	28.9
Mean[b]	0	2.81	3.05	2.72	2.27	2.92	1.97	1.43	1.89	1.50	1.62	88	86	56.7	2	27.3

a Means of infested symptom-free plants.
b Means of uninfested check plants.
c 0 = Symptom-free.
   1 = Symptoms limited to a single inner leaf.
   2 = Typical wilt.

ing between each separate infestation in the second set of 18 plants. There were 18 check plants.

Each time these plants were infested, mealybugs from the same lot were used to infest plants growing in field plots. Ten plants were infested with 10 mealybugs each and 10 with 100 mealybugs each, and these were sprayed out after 10 to 14 days. The first mealybug infestation was made on November 2; the last on January 3. Root elongation data were taken until March 12 and the final leaf symptoms on April 3.

The first comparison to be made is of the relative wilting in the field plots with the two degrees of infestation and in the water vapor boxes with the two types of infestation. None of the field plants infested with 10 bugs each wilted and of the plants infested with 100 bugs each, 26 per cent wilted. There were 4 cases of wilt in the set of 18 plants which were infested with 10 bugs 3 times without intermittent spraying, and all of these developed to the severe form. Of the 18 plants infested three times but with intermittent spraying, 6 showed wilt symptoms but in only one case did these progress beyond a single leaf.

This is an interesting case of the accumulated effects of mealybug feeding in inducing wilt symptoms on pineapple plants. It is clear that wilt occurred following three successive small infestations of mealybugs none of which alone were capable of inducing wilt symptoms, and that intermittent spraying reduced the extent to which symptoms developed. The length of time necessary for the expression of symptoms, both on the roots and on the leaves was much delayed in comparison with the typical sequence as shown in figure 4. In table 9, the data for the two series are presented for each individual wilted plant. This is for the purpose of calling attention to differences between individuals, particularly between those plants whose symptom expression progressed to complete wilt and those with limited symptoms only. In order to reduce tabulated matter, the data for the first nine weeks are omitted, there being no indication until later of any effect on root elongation. The three symptom classes for this experiment are 0, symptom-free; 1, with symptoms limited to a single inner leaf, and 2, typical wilt.

Referring to table 9, it will be seen that all five plants in symptom class 2 showed typical root collapse. One plant, 4C6, was in the recovery stage when the experiment was concluded and its new root system was well developed. Another plant in this class, 5A4, had a fair new root system developed with no evidence of recovery when the experiment was concluded. The cessation of root elongation in this class indicated that some were affected after the second infestation of mealybugs, others after the third.

Plants in symptom class 1 which showed only a single leaf wilted, varied considerably in their root elongation sequences. Two of them, plants 5A2 and 5B3, showed evidence of two depressions in root elongation, one in the tenth week following the first infestation and another several weeks later.

In all the plants in this class there was more than half the root mass alive at the end of the experiment. It can also be observed that root elongation generally, including that of the symptom-free infested plants, was depressed in the plants which had been sprayed with oil emulsion three times during the course of the experiment.

SUMMARY

When pineapple plants are grown in water vapor cabinets, it is possible to follow the symptom sequence of mealybug wilt with considerable accuracy. The first measurable symptom is the cessation of root elongation. This is followed by death of the roots which usually but not always involves the entire root mass before wilting of the leaves occurs. The first recovery symptom is the production of new roots and this is followed by the restoration of turgor to the central leaves of the plant which proceed to normal growth.

Sub-wilting symptoms, recognizable only by detailed plant growth measurements, sometimes occur and are expressed as reduction in plant growth.

Tests with planting pieces from the same mother plant revealed differences between sister pieces both in susceptibility to wilt and in subsequent development of symptoms. Tests with differential feeding of green spotting and non-green spotting strains of mealybug revealed that free living mealybugs were more effective than those restricted in their feeding area by caging. This was particularly true of the non-green spotting strain.

Continuous infestations maintained by monthly applications of small numbers of mealybugs resulted in more extreme symptom expression than when similar infestations were sprayed out after two weeks feeding.

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LEAF VARIEGATION IN TUNG

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Leaf variegation in tung (*Aleurites fordi* Hemsl.) was first observed in approximately 75 seedlings in a nursery near Cairo, Georgia, in 1930.

The predominant abnormal color in the older leaves was yellow, but there were three or four shades of yellow, and mixtures of yellow and green. Some leaves appeared normal except for an almost imperceptible spot of yellow, perhaps no more than 2 to 3 mm. wide. In other leaves the yellow coloration was extensive, involving one-half to three-fourths of the leaf area. Within the yellow areas there would often be islands, of various sizes and shapes, of normal green tissue. Uniformity or regularity in pattern of colors, such as confinement to the leaf perimeter or to the central area, were not observed (Fig. 1).

The yellow colors showed only faintly on the under surface of the leaves. The color markings were always present on very young affected leaves, but they showed faintly as a greenish yellow.

This striking tung leaf variegation, when it was first observed, was suspected of being due to some genetic disturbance. The possibility of a virus association was not entirely ruled out, although virus infection of woody seedling plants rarely, if ever, is apparent naturally during the first summer's growth in a nursery.

In November, 1930, tung seedlings possessing variegated leaves were marked for later use and observation. Apparently healthy seedlings were also marked. Buds taken from variegated-leaf plants were budded by the ring-bud method into the base of 10 normal seedlings. Buds taken from normal-leaf plants were placed in 10 variegated-leaf plants. Young variegated leaves and succulent shoot tissues taken from affected seedlings were crushed with a small amount of water in a mortar. This liquid was then injected with a hypodermic syringe into buds and young portions of 10 healthy seedlings.

All marked and treated seedlings were transferred from the tung nursery near Cairo, Georgia, to the U. S. Pecan Station, Philema, Georgia, in January, 1931, and planted 12 feet apart in semi-nursery formation. The following spring the budded trees were cut back to about 1 inch above the implanted bud. This plot of tung trees was under observation through the following 5 growing seasons.

The final records showed that trees possessing variegated leaves, while growing in the nursery rows in 1930, continued to form variegated leaves throughout the period of the experiment. Trees that were normal in the

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beginning continued in their normal status. Buds taken from variegated-leaf trees and placed in normal seedlings produced shoots with variegated leaves. Adventitious shoots that grew from points below the implanted buds formed normal-colored leaves. Buds taken from normal seedlings and implanted in variegated-leaf trees produced shoots bearing normal-colored leaves. And trees injected with sap expressed from leaves and succulent tissues of variegated-leaf seedlings produced only normal green leaves throughout the period of the experiment.

In 1936, twenty of the young variegated-leaf trees growing in semi-nursery formation at Philema, Georgia, blossomed and produced a few nuts as the result of open pollination. These were harvested, nuts from each tree being placed in a separate bag, stored during the winter, and planted the following spring.[3]

FIG. 1. Tung leaf with typical pattern of variegation. About ⅓ natural size.

Two hundred and sixteen seedlings grew; of these, 114, or 52.8 per cent, were of the variegated-leaf type. Some variegated-leaf trees produced seeds forming only green-leaf seedlings. Seeds of other trees produced seedlings with leaves only of the variegated type. On the other hand, seeds from some trees formed both green-leaf seedlings and variegated-leaf seedlings in various proportions.

While the number of seedlings giving rise to variegated leaves in comparison to the total number grown was adequate to demonstrate the transmissibility of the variegation character through the seed, the number of seedlings from individual trees was too small to be of any statistical importance for forecasting the pattern of inheritance. Some trees produced as few as two to five nuts, although seven trees bore 15 to 25 nuts each.

The evidence presented suggests the involvement of an inheritance factor

rather than the presence of an infection agent, such as a virus. The variegated leaf character was not transmitted (1) by injecting expressed sap from affected shoots, leaves, and buds into healthy tree tissues; or (2) through the medium of tissue grafts. Buds taken from green-leaf trees formed only green-leaf shoots when implanted into trees having variegated leaves. Buds taken from variegated-leaf trees produced shoots with variegated leaves and the character of variegation did not migrate to adjacent tissues of the stock.

The inheritance of this tung variegation appears to be genetic in type. If it were cytoplasmic, or maternal, then all seedlings growing from variegated trees should have variegated leaves. This did not occur. Four trees produced only variegated seedlings, 2 produced only green seedlings, and nuts from 14 others gave both types of seedlings.

Normal appearing tung trees have been known to transmit variegation to their progeny. Nuts of the Fairchild variety persistently produce some variegated seedlings. This variety originated from a single-leaf seedling, planted in 1930 in the J. B. Wight Nursery, Cairo, Georgia, where the first specimens of tung variegation were observed that same year. This planting was made with seeds secured in small lots from several sources in south Georgia and north Florida. A portion of the seeds came from single isolated tung trees serving for shade around farm homes. Undoubtedly some of those single trees grew from the earliest lots of tung nuts introduced into the United States from China during the first decade of this century, or from seedlings distributed by the United States Department of Agriculture. Mr. Wight received one seedling in 1908, and seeds from this tree were used in part for planting the nursery·in 1930.

We can only speculate as to the source of the tung seeds that produced the variegated-leaf plants. There is a possibility that they all came from a single tree, because, although the variegated-leaf plants were scattered, all were in a restricted section of the nursery.

SUMMARY

Although leaf variegation in tung (*Aleurites fordi* Hemsl.) is very infrequent and not important economically, trees possessing a variable leaf pattern composed of three or four tones of green and yellow occasionally appear.

The earliest observation of this phenomenon was in a tung nursery near Cairo, Georgia, in 1930. It was suspected that the abnormal leaf coloration either was associated with the presence of a virus or that some genetic abnormality was involved.

Among 216 seedlings grown from open-pollinated seed produced by 20 abnormal-leaf trees, 114 or 52.8 per cent, were of the variegated-leaf type.

The evidence presented suggests the involvement of an inheritance factor rather than the presence of a virus.

PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND,
       AND
LABORATORY FOR TUNG INVESTIGATIONS, BOGALUSA, LOUISIANA.

THE EFFECT OF PRE-PLANTING IRRIGATION ON PATHO-GENICITY OF RHIZOCTONIA SOLANI IN SEEDLING COTTON

GLEN STATEN AND JAMES F. COLE, JR.[1]

Damping-off or soreshin of cotton caused by *Rhizoctonia solani* Kühn is well known, especially in the irrigated areas of the Southwest where the organism occurs generally, attacking cotton and other crops. While much investigational work with seed treatment has been done in an attempt to control the disease, there has been little offered on cultural control.

Lehman[2] has called attention to the fact that the organism in an infested soil is very virulent soon after inoculum is added, but rather quickly loses this extreme virulence. A commonly suggested explanation for this phenomenon is an increase in population of antagonistic or competitive organisms which reduce the virulence of *Rhizoctonia*.

FIELD EXPERIMENT

A field experiment at State College, New Mexico, yielded as a by-product information which suggested that partial control of the disease is possible. The experiment was the conventional two-factor experiment with nine randomized blocks, two types of seedbeds, flat and double-row bed, combined with two quantities of irrigation water, light and heavy, beginning with the initial irrigation. In order to follow accepted farm practice for the locality, it was necessary to irrigate the double-row bed type of seedbed three weeks prior to planting, and the seed was planted in moist soil. With the flat seedbed, the seed was drilled in the dry soil and moisture supplied by an irrigation after planting.

A severe outbreak of *Rhizoctonia* occurred in some of the plots, one to two weeks after emergence of the seedlings, at which time the plots had been given only the initial irrigations. Counts of post-emergence damping-off were made and a summary of the results are in table 1 with an analysis of variance of the data in table 2. No significant differences were obtained between heavy and light irrigations. However, within each of these groups significant differences were secured. Since the experiment was planned for another purpose, the damping-off counts being merely a by-product, the effects of kind of seedbed and pre-planting versus no pre-planting irrigation were confounded and could not be separated. Since it did not seem reasonable to assume that type of seedbed should affect the pathogenicity of a soil-borne fungus, it was assumed that the differences in the virulence of the organism were due largely to the pre-planting irrigation rather than to seedbed effect.

[1] Associate Agronomist and Assistant in Agronomy, respectively.
[2] Lehman, S. G. Cotton seed dusting in relation to control of seedling infection by *Rhizoctonia* in the soil. Phytopath. **30**: 847–853. 1940.

GREENHOUSE EXPERIMENT

A second experiment was set up in the greenhouse with 13 replicates of 4 treatments in 1-gallon stone jars. Treatments were field soil with and without inoculum added, each combined with pre-planting and no pre-planting irrigation. Field soil was passed through a 4-mm. screen and in the infested series a virulent inoculum was worked into the surface inch of soil. In order to simulate field conditions, the inoculum was prepared by gathering from the field, cotton seedlings which had been killed by

TABLE 1.—*Effect of light and heavy irrigations before planting and at planting time on occurrence of post-emergence damping-off in field experiment*

Treatment	Mean percentage post-emergence damping-off
Light irrigation	
Flat seedbed with no pre-planting irrigation	11.69
Double-row bed with pre-planting irrigation	2.23
Heavy irrigation	
Flat seedbed with no pre-planting irrigation	14.50
Double-row bed with pre-planting irrigation	4.57

Least significant difference at odds of 19:1 is 7.11.

TABLE 2.—*Analysis of data from field experiment on occurrence of damping-off*

Source of variation	Degrees of freedom	Mean squares
Replicates	8	34.45
Heavy vs. light irrigation	1	59.55
Within heavy irrigation group	1	444.02**
Within light irrigation group	1	402.33*
Error	24	53.44

* Probability value less than 0.05.
** Probability value less than 0.01.

Rhizoctonia, grinding and mixing the air dried plants and adding 2.5 gm. of this inoculum to each pot of soil. Pure cultures of the organism were secured from the dead cotton seedlings.

The experiment was begun June 7, the soil in half the pots being kept at optimum moisture content from that time until planting date, June 30, the other half remaining dry. Nineteen acid-delinted, Ceresan-treated seed of the Acala 1517 variety were planted in each pot. Daily counts on emergence and damping-off were made until the termination of the experiment, July 30, at which time all surviving plants were examined for lesions.

A summary of the results of this experiment is in table 3 with analyses of variance of the results in table 4. Since the data were of the discrete type, analyses were made with the original data expressed both in percentages and after transforming to degrees of an angle. Since both types of analyses yielded the same results, the analysis presented is from the data expressed in percentages. Because a significant interaction was not ob-

TABLE 3.—*Summary of greenhouse experiment on effect of pre-planting irrigation on occurrence of damping-off*

Data	Field soil with no inoculum added			Field soil with inoculum added		
	No pre-planting irrigation	Pre-planting irrigation	Differ-ence	No pre-planting irrigation	Pre-planting irrigation	Differ-ence
tage emergence	87.85	81.79	6.06	78.12	72.88	5.24
tage post-emer-e damping-off	2.43	.48	1.95	40.98	26.28	14.70*
tage lesion-free ts surviving ..	68.13	89.21	21.08*	6.27	15.46	9.19

* Significant differences, P less than 0.05.

tained in any case, the more informative analysis appears to be the between and within group type.

In percentage emergence there was no significant difference between pre-planting versus no pre-planting irrigation. There was a significantly lower percentage emergence from the infested soil than from the soil receiving no inoculum, even though Ceresan-treated seed were used. In the noninfested soil, very little post-emergence damping-off occurred in either the pre-irrigated series or the one not pre-irrigated. In the soil group with inoculum added, however, the pre-planting irrigation treatment significantly lowered the percentage of post-emergence damping-off from 40.98 to 26.28.

In the group receiving no inoculum there was a significant difference in the percentage of lesion-free plants surviving in favor of those grown in soil receiving the pre-planting irrigation. The series in this group receiving no pre-planting irrigation produced a considerable number of lesions even though the mortality was negligible. In the soil with inoculum added lesions were produced on the greater portion of the plants

TABLE 4.—*Analyses of variance of data from greenhouse experiment on occurrence of damping-off*

Sources of variation	Degrees of freedom	Mean Squares		
		Percentage emergence	Percentage post-emer-gence damp-ing-off	Percentage lesion-free plants surviving
Replicates	12	129.75	313.32	247.15
Soils: Inoculum added vs. no inoculum added	1	1129.95**	13456.39**	60295.74**
Within soil groups: Inoculum added	1	177.85	1403.12*	549.24
No inoculum added	1	238.83	24.62	3054.28*
Error	36	130.19	205.77	461.64

* Probability value less than 0.05
** Probability value less than 0.01.

whether receiving a pre-planting irrigation or not. The difference in the amount of disease was also evident in the growth of the plants. The plants on the noninfested soil receiving a pre-planting irrigation were outstanding for size probably because of the high percentage of lesion-free plants.

The experimental results reported herein check with field observations made in the irrigated areas of the Southwest where severe infestations of *Rhizoctonia* have been noted to occur more commonly when seed of susceptible crops are planted in dry soil after such crops as alfalfa and sugar beets grown for seed. In such cases, the inoculating material consists of dry infected plant refuse which produces severe damping-off.

CONCLUSIONS

Experimental results indicate that a partial control of *Rhizoctonia* can be secured by giving the seedbed a pre-planting irrigation and thereby allowing an incubation period of several weeks duration. The results suggest that the pathogen is partially inhibited by antagonistic or competitive organisms when the soil is given a pre-planting irrigation.

In rainfall areas early seedbed preparation when soil moisture is present is suggested for partial control of disease.

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THE COLORIMETRIC DETERMINATION OF 2,3-DICHLORO-1,4-NAPHTHOQUINONE ON SEED

H. P. BURCHFIELD AND G. L. MCNEW [1]

A colorimetric method for determining the dosage of tetrachloro-p-benzoquinone on seed was described (1) in a previous publication. Since another fungicidal member (7) of the quinone series, 2,3-dichloro-1,4-naphthoquinone,[2] has been used extensively for the treatment of spinach and beet seed (4) and has shown distinct promise as a protectant for seed of rice (2), peanuts (8), pepper, tomato, maize (3), sorghum (5), and other crops (3, 6), there is need for a comparable, simple method of determining the dosage of this fungicide. Although the method developed for tetrachloro-p-benzoquinone did not prove directly applicable, a modified colorimetric method of analysis was developed. This procedure and data on its reliability for measuring the dosage of three different grades of dichloronaphthoquinone on several types of seed are presented in this paper.

CHEMICAL REACTIONS

The most convenient method of removing the chemical from seed for analysis is to dissolve it in a selective organic solvent that also provides a suitable reaction medium. Acetone was found to be the most satisfactory. The reactions of dichloronaphthoquinone in this solvent with aqueous sodium hydroxide, trimethylamine, and diethylamine were investigated. Intense colors were produced by all of these reagents but they either formed so slowly or faded so rapidly that they were not entirely suitable for photometric analysis. In the presence of 10 per cent aqueous dimethylamine, however, an intense red color developed immediately and remained stable for at least 15 minutes. On longer standing, it faded to a pale yellow that exhibited green fluorescence upon dilution with water.

The exact structure of the red reaction product is not known. Presumably, an unstable intermediate complex of dimethylamine and acetone with dichloronaphthoquinone is formed. The formation of 2-dimethylamino-3-chloro-1,4-naphthoquinone would normally be anticipated since this material is obtained in good yield when the reaction is carried out at high concentrations. Although these two materials exhibit maximum absorption (Fig. 1) in the visible range at 495 mμ, the specific extinction coefficient of the red complex, calculated on the weight of the dichloronaphthoquinone, is more than twice that of the dimethylamino derivative.

Regardless of the chemical nature of the red compound, it was found to

[2] Sold commercially under the trade-name of Phygon and tested extensively since 1942 under the code U. S. Rubber Compound 604.

be well suited for the colorimetric determination of dichloronaphthoquinone. The color formed quickly, was sufficiently stable for routine analysis and was proportional in intensity to the concentration of fungicide in solution.

MATERIALS AND METHODS

In order to standardize the available equipment and determine the analytical constant for pure dichloronaphthoquinone, tests were made in a
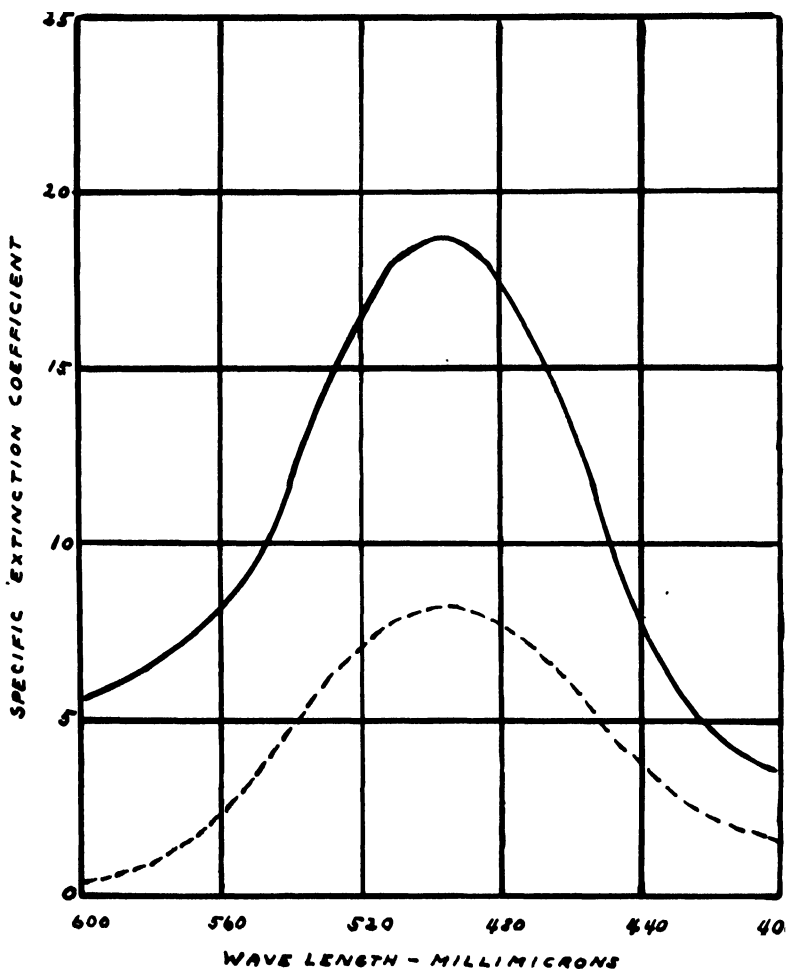
FIG. 1. Absorption spectra of the reaction product of dichloronaphthoquinone with dimethylamine and acetone (solid line) and of 2-dimethylamino-3-chloro-1,4-naphthoquinone in acetone (broken line).

Lumetron colorimeter with several concentrations of a purified sample (m.p. 193° C.) recrystallized from acetic acid. An acetone solution containing 0.25 gm. per liter was prepared and aliquots of 25, 20, 10, 5, and 2 ml. each were transferred to 50-ml. volumetric flasks containing 10 ml. of 10 per cent aqueous dimethylamine and sufficient acetone to make a total volume of ap-

proximately 45 ml. after adding the aliquot. The mixtures were made to volume with acetone and transferred to a 1-cm. absorption cell for photometric analyses in a colorimeter equipped with a green filter.

When the data on the percentage of light transmitted (T) by the various samples were converted to optical densities and plotted against the concentration of dichloronaphthoquinone (grams per liter in the final test solution), a linear relationship was found. The apparent specific extinction coefficient, calculated as described elsewhere (1) was found to be 14.6 with the equipment used. Repetition of the test showed that the results were reproducible to within ± 1.10 per cent,of the total chemical provided the above procedure was followed. Changes in the order of adding the reagents produced small, but significant, differences in the intensity of the color.

For the analysis of seed, 5-gram samples were weighed to the nearest 0.01 gm., washed in three or four portions of acetone (analytical reagent grade), and the combined extracts made up to 50 ml. After filtration through a fluted No. 5 Whatman paper to remove foreign material, an aliquot of 25, 10, 5, or 2 ml. depending upon the dosage anticipated, was transferred to a 50-ml. volumetric flask containing a dimethylamine-acetone mixture and treated as described above. The percentage of dichloronaphthoquinone on the seed was calculated as follows:

$$\text{Percentage of dichloronaphthoquinone} = \frac{5 \, Vo \left(\log \frac{100}{T} \right)}{KVW}$$

where Vo is the initial volume of the extract, V the volume of the aliquot, K the apparent specific extinction coefficient, and W the sample weight.

In order to measure the reliability of this method, seed of hybrid dent corn and peas of the variety Perfection were treated with three different formulations of dichloronaphthoquinone (Phygon, about 87 per cent active; Phygon-DDT-S1, a wettable grade about 85 per cent active and containing 1 per cent of DDT; and a special mixture containing 50 per cent of active material in whiting). Each composition was added to glass jars containing approximately 200 gm. of seed weighed to the nearest 0.01 gm. Sufficient material was added to obtain a dosage of 0.48, 0.24, 0.12, 0.06, and 0.03 per cent by weight after a nominal loss on the walls of the container. After the seed and chemical had been tumbled for 30 minutes on a vertical turntable at 28 rpm, the seed were reweighed, correction was made for any loss or gain in the weight of the untreated check, and the dosage of chemical was calculated.

Five-gram samples from each treatment were analyzed in triplicate by the above method and the results corrected for the percentage of active ingredients in each formulation. Emergence data were taken on 16 replicates of 25 seeds each from these lots sown in composted greenhouse soil that was known to be severely infested with seed-decaying fungi. The soil was thoroughly moistened and held at 55° F. for seven days after the seed was sown in order to induce severe seed decay.

EXPERIMENTAL RESULTS

The data obtained on corn and pea seed treated at different dosage levels with the three formulations of dichloronaphthoquinone are given in table 1. The analytical data (mean of three determinations) are in very close agreement with the calculated dosages obtained by weighing. The discrepancies ranged from 0 to 12 per cent at the higher dosages and from 0 to 90 per cent at the lower dosages.

The colorimetric method is inherently as accurate at the lower dosages as at the higher ones since the concentration can be adjusted to the optimum

TABLE 1.—*Relationship of dosage on pea and corn seed as determined by weighing and colorimetric analyses to emergence in infested soil*

Treatment applied to seed	Results on corn seed			Results on pea seed		
	Dose by weighing	Dose[a] by analysis	Emergence	Dose by weighing	Dose[a] by analysis	Emergence
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Phygon	0.47	0.488	93.0	0.44	0.418	94.2
	0.26	0.248	90.0	0.21	0.215	93.8
	0.12	0.121	89.8	0.12	0.124	88.8
	0.08	0.064	83.5	0.05	0.056	81.2
	0.02	0.037	79.8	0.04	0.037	83.5
Phygon—DDT	0.44	0.485	87.6	0.41	0.413	93.5
	0.26	0.262	90.5	0.21	0.219	92.5
	0.12	0.158	87.8	0.15	0.159	93.8
	0.06	0.075	87.5	0.07	0.075	83.8
	0.03	0.059	78.5	0.05	0.058	82.2
Phygon—50 per cent	0.46	0.417	85.3	0.42	0.368	89.5
	0.23	0.228	84.0	0.20	0.208	85.8
	0.14	0.127	85.0	0.15	0.149	88.0
	0.06	0.060	78.5	0.07	0.062	74.8
	0.02	0.033	64.3	0.03 −	0.064	63.2
None	0.00	0.000	16.0	0.00	0.000	37.5
	0.00	0.000	18.8	0.00	0.000	37.5
	0.00	0.000	18.0	0.00	0.000	40.5
Minimum Sign. Diff. 5 per cent			5.2			7.1
Minimum Sign. Diff. 1 per cent			6.8			9.3

[a] Corrected to 100 per cent on basis of percentage active ingredients.

by dilution; so these differences at the lower dosages suggest that most of the error is in the weighing method. A statistical analysis of the triplicate colorimetric tests showed a mean range of 4.3 per cent of the total chemical present, and a standard deviation of 2.53 per cent for the individual analyses. Since the standard deviation of the analytical constant on pure chemical in known concentration was 1.10 per cent, the error attributable to sampling and weighing the seed was in the order of 2.28 per cent of the total dichloronaphthoquinone on the seed.

It is obvious that the analytical data are much more reliable than those from either weighing or seedling emergence. The slopes of the dosage-response curves on both crops were not sufficiently steep to be of practical use in measuring small variations in the amount of chemical applied. The

weighing method is inaccurate because it is based on the measurement of small differences (0.01 to 0.04 gm. of chemical) between two large masses (200 gm. of seed). The only analytical data that might be construed as being unreliable are those obtained on peas with 50 per cent Phygon at 0.03 per cent. Both the seedling emergence and dosage by weight indicate a significantly lower dosage than in the preceding treatment. This one discrepancy is minor, however, in view of the other 29 observations.

Less extensive tests were made on seed of Lima beans (var. Fordhook), spinach (var. Virginia Savoy), and beets (var. Asgrow Canner). Seed were treated at recommended rates of 0.25, 0.50, and 1.00 per cent, respectively. By reweighing the seed, the dosages were calculated to be 0.22, 0.60, and 1.05 per cent by weight. The means of triplicate colorimetric determinations for the three crops were 0.216, 0.573, and 1.03 per cent, respectively. In making these tests it was found that a 20-gm. sample of Lima bean seed gave much more reliable results than a 5-gm. sample while 2.5 gm. of either spinach or beets gave accurate records. When making tests on samples of these different sizes the seed were washed in acetone and made to the customary volume of approximately 10 ml. per gram of seed.

SUMMARY AND DISCUSSION

A simple, rapid method of analyzing seed for dosage of 2,3-dichloro-1, 4-naphthoquinone was developed. The chemical is washed from the seed with acetone and treated with 10 per cent aqueous dimethylamine to produce a red color. The intensity of the color is directly proportional to the amount of dichloronaphthoquinine; so, accurate determinations can be made by measuring the amount of light transmitted. The dosage can be calculated from these data or estimated graphically by plotting the calibration points for known concentrations of the chemical on semilogarithmic paper and fitting the best straight line. The concentration of fungicide in the final solution can be determined directly from this graph.

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PHYTOPATHOLOGICAL NOTE

Influence of Nutritional Level on the Susceptibility of Tomatoes to Fusarium wilt.[1]—Recently Walker and Foster[2] have reported that nutritional balance and total salt concentration affect severity of, as well as susceptibility to, pathogenesis by *Fusarium lycopersici* Snyder and Hansen. These authors conclude that "disease devolopment in susceptible or intermediate resistant hosts grown at a temperature optimum for wilt, decreased with an increase in nutrient concentration regardless of the degree of virulence of the pathogen used or the age of the plants within the limits of the studies."

To produce tomatoes of maximum susceptibility to Fusarium wilt for chemotherapeutic studies, plants of the susceptible John Baer variety were grown in builders' sand in unglazed pots. A complete nutrient solution of the same composition as that used by Walker and Foster (0.1 H) was added at weekly intervals at the rate of 25 ml. per pot, the balance of the water requirement being supplied as water. Such plants, when inoculated with the virulent strain of *Fusarium* used in the Wisconsin studies,[3] became diseased only very slowly if at all. Quite obviously the nutritional level in our tests was lower than the minimal level examined by Walker and Foster since their nutrient solution was supplied in drip culture and constituted the sole source of water for the plant. Apparently, also, the plant expresses an increased resistance to disease with a decreased nutrient concentration below the minimal level examined by these authors.

To test this hypothesis, John Baer tomatoes were grown in builders' sand in pots in the greenhouse, at a thermostatically controlled temperature of 83° F. (28° C.). The same nutrient solution was used as was employed by Walker and Foster, and applied at concentrations corresponding to their 0.1 H, 1 H, and 10 H levels of nutrition. Such nutrient was applied every other day at the rate of 25 ml. per pot, the remainder of the water requirement being supplied as water. Inasmuch as the nutrient solution in our tests was not the sole source of water, the levels of nutrition are less than those used by Walker and Foster and are here specified as 0.1 X, 1 X, and

TABLE 1.—*Effect of level of nutrition on susceptibility to and severity of Fusarium lycopersici in John Baer tomatoes*

Nutritional level	Number infected (of 20 plants)	Percentage of plants infected	Index of severity
0.1 X	8	40	26
1 X	19	95	75
10 X	1	5	0

10 X, respectively. Twenty plants were maintained at each nutritional level for one month before inoculation, and for one month afterwards before readings were taken. Readings of disease were based on (1) the number of plants infected, as judged by the appearance of vascular discoloration and wilted or dying leaves and (2) the severity of disease expression based on indexes of disease as used by the above-mentioned authors. Results are reported in table 1.

Further differences between lots of plants are shown photographically in figure 1.

FIG. 1. Photograph of tomato plants grown on two nutritional levels and inoculated with *Fusarium lycopersici*. The two plants on the left show the severity of disease when plants were maintained at X nutritional level, whereas the two plants on the right indicate the response of plants maintained at the 0.1 X level. Plants maintained at the 10 X level appeared completely healthy and received an index of severity of 0 (See table 1).

There is evidently an optimum nutritional level for infection of tomatoes and severity of disease by *Fusarium lycopersici*. This lies in the region between the minimum for normal growth and the ideal.—E. M. STODDARD and A. E. DIMOND, Connecticut Agricultural Experiment Station, New Haven, Connecticut.

ANNOUNCEMENT

FORTIETH ANNUAL MEETING

The fortieth annual meeting of The American Phytopathological Society will be held at the William Penn Hotel, Pittsburgh, Pennsylvania on December 6, 7, and 8, 1948. The Northeastern Division will meet with the parent Society. The Council will meet on Sunday, December 5, at 9:30 A.M. Accommodations for 375 persons have been reserved by the hotel for our meeting. You are urged to return to the hotel, as soon as possible, the room reservation card which is being mailed to all members.

Arrangements have been made for meeting rooms for the usual sessions of the Society. However, facilities for special group meetings must also be arranged in advance and it will be appreciated if requests for such meetings are forwarded to the Secretary, Curtis May, at the earliest possible date.

In accordance with provisions of the Constitution of the Society, abstracts of papers to be presented must not contain more than 200 words and must be received by the Secretary not later than October 8, 1948. The Constitution directs the Secretary to return, to the author, abstracts submitted after the closing date, which is 60 days prior to the annual meeting.

Anyone interested in tours through industrial plants in the vicinity of Pittsburgh, may write the chairman of the local committee on arrangements, Dr. W. C. Price, University of Pittsburgh. Consideration is being given to arranging such tours for December 9.

FACTORS AFFECTING THE DEVELOPMENT OF BACTERIAL SOFT ROT OF POTATO TUBER INITIALS[1]

R . S . DA V I D S O N [2]

Potatoes grown in low, poorly drained areas produce low yields when excessive rainfall prevails for several days during the period of tuber initial formation. Lenticel infection of tuber initials, similar to that described by Smith and Ramsey (27) on early harvested mature tubers, was observed by the writer during field studies on the incidence of bacterial soft-rot at the Minnesota Agricultural Experiment Station. Bacterial lenticel infection of mature potato tubers, as observed at harvest, has been reported (13, 14, 15, 24, 25, and 26), and the pathological histology and identity of the bacteria involved have been studied (27). This paper deals with the environmental conditions under which lentical infection of tuber initials may occur, the extent of such infection, and the bacterial organisms involved. These studies were conducted at the Minnesota and the Rhode Island Agricultural Experiment Stations.

MATERIALS AND METHODS

At the Minnesota Station, Irish Cobbler potato plants were grown in 8-inch clay pots, in the greenhouse. At the Rhode Island Station, Irish Cobbler and Green Mountain plants were grown in Wagner-type subirrigation pots as described by Wheeler et al. (31) or plants were grown in greenhouse ground beds 48 by 60 inches and 18 inches deep with a gravel underlay.

Soils that had been under a cultivation program which included potatoes in the rotation were used. In Minnesota, sandy loam from University Farm was used; and Bridgehampton very fine sandy loam from the Experiment Station Farm, where potatoes had been grown continuously for at least 5 years, was used in Rhode Island.

In the Minnesota experiments, all fertilizer was applied as follows: 40 lb. N, 240 lb. P_2O_5, and 120 lb. K_2O per acre. The nitrogen was derived from sulphate of ammonia, the P_2O_5 from concentrated superphosphate (43 per cent P_2O_5), and the K_2O from muriate of potash. In the Rhode Island experiments, with the exception of specific studies on the relation between varying amounts of inorganic fertilizer constituents and the occurrence of infection, fertilizers were applied at the following rates per acre: 90 lb. N,

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⅓ derived from nitrate of soda and ⅔ from sulfate of ammonia; 180 lb. P_2O_5, 5/9 of which was derived from superphosphate and 4/9 from concentrated superphosphate (47.5 per cent P_2O_5); 180 lb. K_2O, from muriate of potash. As a safeguard against magnesium deficiency, 30 lb. MgO as calcined magnesite (88.5 per cent MgO) was added.

It was evident from the outset that soil moistures produced in the greenhouse would not be entirely representative of field conditions. Nevertheless, an attempt was made to simulate field conditions in so far as possible. All water added to the soil was on the basis of monthly rainfall equivalent. To obtain a standard for pots and beds the amount of water necessary to approximate a given monthly rainfall rate was determined on the basis of the surface area of an individual pot and a greenhouse bed. Water was added at daily intervals, 0.4 gallon equalling one inch of rainfall for a greenhouse bed and 61 cc. equalling one inch for each Wagner pot. Water was added to the greenhouse beds from overhead and was measured by means of a disc water meter. Water was applied to the surface of the soil in the Wagner pots by direct measurement in cubic centimeters.

Soil moisture in the greenhouse beds and in the Wagner pots was determined on the basis of oven-dry weight, after water had been applied at several established rates to each. Triplicate soil samples for moisture determinations were taken, from a depth of approximately 4 inches, at least every other day, with a butter trier. In the beginning a series of soil moisture determinations was made for Bridgehampton very fine sandy loam; these data were used to compute the amount of water added to the soil in subsequent experiments. On the basis of oven-dry weight determinations, the water-holding capacity of the soil in the beds was approximately 29 per cent, while in the pots it varied between 32 and 35 per cent. Tap water was used throughout the investigations.

STUDIES ON INFECTION OF TUBER INITIALS

The Effect of Soil Temperature

A study was made of the effect of high soil moisture and various soil temperatures on bacterial infection of tuber initials of the Irish Cobbler variety. Constant temperature tanks, described by Christensen (5), were maintained at 4°, 18°, 24°, 27°, 34°, and 39° C.

Each tank accommodated 8 pots, one plant in a pot. Pots were suspended by wire racks attached below the collars of the pots so as to permit complete saturation of the soil with water from the temperature tank, but so as to avoid standing surface water. One potted plant from each of the 7 tanks was removed at approximately 12-hour intervals, the first series of 7 plants being examined 24 hours after the plants were placed in the tanks. The final series of plants was examined 36 hours after the previous series. Each plant was removed from the pot, washed in running tap water, and the number of healthy and infected tubers recorded (Table 1). The experiment was repeated using the same variety and the same soil temperatures. Plants

were examined at 48-hour intervals, beginning 24 hours after the soil was saturated. These data also are recorded in table 1.

Although this study was concerned principally with tuber initials, the response of the plants to environmental conditions also was observed. Blackleg symptoms, as described by Morse (22), appeared only in this phase of the work (Table 1).

No proliferation of lenticels was observed on tuber initials at 4° C. in less than 72 hours. About 60 hours after the beginning of the first experiment, the temperature in the 4° C. tank rose to 10° for about 8 hours because of insufficient ice. Although 4° C. is near the minimum for growth

TABLE 1.—*The relation of soil temperature to bacterial lenticel infection in tuber initials in flooded soils in two experiments*

Soil (°C.) temperature	Time flooded (hours)							
	24	31	47	56	72	80	96	118
	Percentage of tuber-initial infection							
4	0	0	0	0	50	33	.	100
	0		0	..	0	..	100	...
16	0	0	0	0	0	25	.	50
	0		0	.	0	.	100	
21	0	0	83	37	50	100	.	100a
	0	..	62	.....	100	.	100	..
24	0	0	55	100	100	83	....	100a
	25	.	100	. .	100	. .	100	..
27	0	0	100	100	100a	100a	...	100a
	50	.	100	. ....	100	.	100	.
34	29	100	100	100a	100a	100a	.	100a
	68	.	100	.....	100	.....	100a	. .
39	100	100	100a	100a	100a	100a	...	100a
	100		100	..	100	..	100a	. .
Control	0	0	0	0	0	0	.	0
	0	..	0	. ....	0		0	

a Plants with symptoms of blackleg at time of examination.

of the bacterial organisms associated with soft rot, the higher temperature probably increased the bacterial activity in the soil and favored lenticel proliferation, since a slight amount of proliferation was observed in the initials examined after 72 hours. The combination of the higher temperature and lenticel proliferation probably favored entrance of the bacteria. When the original temperature was restored, periderm formation probably was retarded enough to prevent normal development and maturation of the periderm cells. Therefore, they were not resistant to the dissolving enzymes produced by the bacteria. The lenticels of potato tubers proliferate very little at low soil temperatures according to Devaux (8). He found that the optimum for proliferation was between 20° and 30° C. This was true also in the present study, where lenticel proliferation increased as the temperature increased within the 20° to 30° C. range.

The low percentages of infected tuber initials at 21° C. (Table 1) with

56 and 72 hours' exposure in moist soil may have been due to the complete disintegration of a portion of the infected initials, thus making an accurate count of total number of infected tubers impossible.

The Effect of Soil Fertility

An experiment was conducted to determine the effects of different ratios of nitrogen, phosphorus, and potassium on lenticel infection in saturated soils. The standard fertilizer treatment was a 6–12–12 grade. The following 6 modifications of this standard, as well as an unfertilized treatment, were included: 0–12–12, 6–0–12, 6–12–0, 12–12–12, 6–24–12, and 6–12–24.

Each fertilizer grade was applied to 24 individual Wagner pots; 12 were planted with a single seedpiece of Irish Cobbler variety and the remain-

TABLE 2.—*The percentage of tuber initials of Irish Cobbler and Green Mountain varieties infected through the lenticels in soils saturated for different lengths of time and fertilized with different grades*

Variety and flooding time	Fertilizer grade applied at time of planting							
	0–0–0	0–12–12	6–0–12	6–12–0	6–12–12	12–12–12	6–24–12	6–12–24
	Infected tubers (per cent)							
Green Mountain								
Flooded 96 hrs. ...	48	30	63	52	39	66	52	50
120 hrs. ...	28	54	75	60	43	46	43	63
144 hrs.	68	44	67	51	62	61	65	60
168 hrs. .	45	55	57	54	72	59	81	78
Average[a]	47	46	65	54	54	58	60	63
Irish Cobbler								
Flooded 96 hrs.	36	48	71	52	67	56	54	41
120 hrs. . .	64	52	62	63	45	65	51	34
144 hrs. .	44	50	71	37	59	43	60	37
168 hrs. . .	53	41	75	57	55	83	65	53
Average[a] . .	49	48	70	52	56	62	58	41
Average both varieties[b]	48	47	68	53	55	60	59	52

[a] Difference necessary for significance at 5 per cent level is 15.6 per cent.
[b] Difference necessary for significance at 5 per cent level is 11.5 per cent.

ing 12 with Green Mountain variety. The various fertilizer combinations were mixed thoroughly with the top 4 inches of soil in each pot before planting. Twenty-four pots, 12 of each variety, were prepared without fertilizer treatment. The planted pots were placed in the field during the last week in May with treatments randomized within the variety. The pots were protected from all natural rainfall. Throughout the early part of the growing period the soil moisture in all pots was maintained at 20 to 22 per cent on oven-dry basis. Seventy days following planting, the moisture content of the soil was increased to 33 to 35 per cent, the approximate field capacity of the soil, and was maintained for 168 hours. The plants were removed from 24 pots, 12 each of the 2 varieties, after 96, 120, 144, and

168 hours. Roots and tuber initials were washed thoroughly in running tap water. The number of tuber initials per plant, the number of infected initials, the degree of lenticel proliferation, and the condition of the seed-piece were recorded.

The percentage of infected tuber initials for each plant was calculated and the data treated statistically according to the recommendations of Hayes and Immer (16). Before an analysis of variance was made, the actual percentages were converted to degrees, as suggested by Bliss (3). The differences in percentage of infected tubers in the different fertilizer treatments were significant at the one per cent level. There was no significant difference between varieties or between any of the first or second order interactions. The average percentages of tuber initials infected in 3 replicates of each treatment for each of the 2 varieties are in table 2.

The Effect of Atmospheric Humidity

Irish Cobbler and Green Mountain plants were grown in Wagner pots in the greenhouse at 18° C. The pots were weighed daily and soil moisture

TABLE 3.—*The percentage of tuber initials of Irish Cobbler and Green Mountain varieties infected at different soil moistures and relative humidities of the air*

Variety	Percentage soil moisture on dry weight basis	Percentage relative humidity of atmosphere	Percentage of tuber initials infected after the number of hours indicated					
			48	95	120	144	168	192
Cobbler	35	85	0	0	25	23	29	55
	35	30	0	0	0	0	0	0
	22	85	0	0	0	0	0	0
	22	30	0	0	0	0	0	0
Green Mountain	35	85	0	50	20	60	45	25
	35	30	0	0	0	0	0	0
	22	85	0	0	0	0	0	0
	22	30	0	0	0	0	0	0

maintained at 22 per cent. Fifty-two days after planting, the soil moisture in half of the pots of each variety was increased to 35 per cent. One half of the pots of each variety at each of the two moisture levels was placed in a Wardian chamber. Two-inch metal standards for the pots kept them above the water that filled the metal pan to a depth of one inch. The relative humidity of the atmosphere in the chamber was about 85 per cent. The remaining lots of potted plants were kept in the greenhouse at a relative humidity of approximately 30 per cent. Daily greenhouse temperatures varied between 19° and 21° C. throughout the 192-hour period. Individual plants from the 4 groups of each variety were examined at 24- to 48-hour intervals, beginning 48 hours after the increased soil moisture and high relative humidity were provided. The results, in table 3, are based on the percentage of infected tuber initials present under the stated conditions of soil moisture and atmospheric humidity. Lenticel infection of tuber initials of

both varieties was found only on plants grown in saturated soil and in an atmosphere with a relative humidity of 85 per cent.

Relation Between Soil Moisture and Lenticel Behavior

According to Hayward (17), lenticels are formed by a division of the cortical cells which produces a group of loosely organized cells, termed complementary cells. Pressure on the complementary cells, resulting from development and growth of the periderm, causes a rupture of the epidermis at the original stomatal opening. Lenticel formation usually results from a local cellular proliferation with the formation of a channel or opening, according to Devaux (9). He also states that a lenticel is an organ in the process of continual growth and destruction. In the potato tuber, the process of continual growth and destruction maintains a state of equilibrium when soil moisture conditions are optimum for potato plant growth. This natural balance between growth and destruction is accompanied by suberization. In wet soils, the rate of growth exceeds the rate of destruction and suberization of the complementary cells, thus creating openings through which soil-borne microorganisms enter.

It is common knowledge that lenticels swell and proliferate when potato tubers are grown in, or exposed to, high soil moistures. De Bary (7) demonstrated that cells formed by the meristematic layer of cells of the lenticels have "hygroscopicity," a tendency to take up water and become swollen. He showed that much of the conspicuous extrusion of the complementary cells which characterizes proliferated lenticels resulted from the swelling of these cells. He also observed that very little proliferation of lenticels occurred at low temperatures and determined the optimum for proliferation to be 20° to 30° C. According to Olufsen (23), moderate soil moistures are the most essential requirement for periderm formation, while excessive soil moistures hinder periderm formation and cause cell proliferation.

Most of the lenticels on the surface of tuber initials were in the apical quarter or third of the tuber. On mature tubers the lenticels were more evenly distributed, with a slight preponderance at the extreme apical end. Lenticel distribution on immature tubers was not always apparent to the naked eye because lenticels were very small and inconspicuous in the apical region. Stomata were found in various stages of transformation to lenticels, progressing from the apical end toward the stem end of the tuber. Developing lenticels were much smaller, less swollen, and less proliferated in the apical region than near the stem end. The extent of the proliferation appeared to vary directly with increasing amounts of soil moisture.

In the present study the bacteria entered the tuber initials through the lenticels in all instances, so far as could be determined. On tuber initials in soil saturated to field capacity, infection is likely to be most abundant near the apical end. The first signs of lenticel infection were most frequent in the apical third of the tuber initial, although lenticels near the stem end

occasionally became infected first. When lenticels in the stem end of the tuber became infected first, the progress of the infection was slow and did not become so extensive as that in the apical region.

Infections through lenticels resulted in very uniform, circular, water-soaked areas from 1 mm. to as much as 20 mm. in diameter. The periphery was usually distinct and uniform. On freshly dug tuber initials the infected spots appear water-soaked and sometimes slightly brown. After exposure to the air for an hour or more, the infected areas become deep pink, and after 24 to 48 hours' exposure to the air, they become brown with a sunken pox-effect on the surface.

The depth of the infection appeared to depend on the age of the tuber initial and age of the infection. In general, the depth was governed by the thickness of the cortex of the tuber initial. The cortex is proportionately thicker in initials than in mature tubers. On tuber initials 15 to 20 mm. in diameter, the depth of the infected area is about equal to the radius of the infected area on the surface. When the infected area on the surface was 10 to 12 mm. in diameter, the depth of the infected areas extended beyond the cortical region. Infection spots often coalesced to form large surface spots, with rather deep penetration into the tuber tissues.

The epidermis covering a single infected lenticel was often distended, forming a blister over the infected area. When freshly dug tubers with blisters were submerged in water, gas bubbles issued from the lenticels. A cracking sound, as the bursting of a small balloon, was detected when pressure was applied to the blisters. Occasionally the entire contents of the tuber decayed, the epidermis merely providing a bag for the watery contents. Once a lenticel became infected, proliferation ceased.

The infected cortex was watery to viscous. The epidermis over infected lenticals was broken easily and often could be washed away with running tap water. The decayed tissue beneath the epidermis was readily washed away also, leaving a uniform crater-like pit. When the epidermis covering several adjacent infected lenticels was removed, the exposed tissue appeared as if chewed by a rodent.

The odor of the decayed tissue was variable. Some infected lenticels, when carefully lanced with a scalpel, were odorless; others had a very strong sour odor, especially those in advanced stages of decay and those resulting from coalescence of several infections. Secondary putrifying organisms may be responsible for the odor in many instances.

Bacteria were consistently isolated from infected lenticels. Several hundred isolations were made, but fungi were never obtained. Actinomyces appeared in some dilution plates but never without bacteria.

Infected lenticels were observed occasionally on the stolons. The only instance in which affected stems were found was during the studies of the effect of various soil temperatures (Table 1). There were many infected initials on plants with blackleg symptoms; however, no association between these two pathologic responses of the host was established in the present study.   .

TABLE 4.—*Physiological characteristics of the cultural types within four species of bacteria isolated from infected lenticels and other parts of potato plants*

Cultural types[a, b]	Physiological tests[c]														
	Gelatin	Starch	M.R.	V.P.	Nitrate	H₂S	Xylose	Dextrose	Mannose	Sucrose	Lactose	Maltose	Glycerol	Salicin	Aesculin
Erwinia carotovora															
1	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	A	AG	−
2	−	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	AG
3	−	−	+	+	+	+	AG	AG	AG	AG	AG	AG	AG	AG	AG
4	−	−	−	+	⊦	−	AG	AG	AG	AG	Ag	AG	A	AG	−
5	−	−	−	⊦	+	−	AG	AG	AG	AG	AG	AG	a	AG	AG
6	−	·	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	ab
7	−	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	−
8	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	AG	AG	AG
9	−	−	+	−	+	+	AG	AG	AG	AG	AG	AG	AG	−	−
10	+	⊦	−	+	+	−	AG	AG	AG	AG	Ag	AG	A⋅	AG	AG
11	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	A	AG	AG
12	−	−	⊦	−	+	−	AG	AG	AG	AG	Ag	AG	A	AG	AG
13	−	−	−	+	⊦	+	AG	AG	AG	AG	AG	AG	AG	AG	AG
14	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	a	AG	AG
15	+	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	−
16	−	−	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
17	⊦	−	−	⊦	+	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
18	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	A	AG	a
19	+	−	−	+	⊦	−	AG	AG	AG	AG	AG	AG	AG	AG	A
20	⊦	+	−	+	+	−	AG	AG	AG	AG	A	AG	a	AG	A
21d	+	−	−	+	+	−	AG	A	AG	A	Ag	a	a	A	A
22d	⊦	−	−	⊦	⊦	−	AG	AG	AG	AG	AG	a	A	Ag	A
23d	⊦	−	−	⊦	⊦	−	Ab	Ab	Ab	Ab	Ab	a	A	Ab	A
E. aroideae															
24	+	−	⊦	⊦	⊦	−	a	A	A	A	A	A	A	−	−
25	+	−	−	−	−	−	a	−	a	−	−	−	−	−	−
26	+	−	−	−	+	−	a	a	a	−	−	−	a	a	a
27	−	−	−	−	−	−	a	a	a	a	a	a	a	a	a
28	+	−	−	−	⊦g	−	a	A	a	−	a	−	a	−	−
29	−	⊦	−	−	−	−	a	A	A	a	a	a	−	a	a
30	+	⊦	−	−	⊦	−	−	−	−	−	−	−	−	−	−
31	−	−	−	−	−	−	A	A	a	−	a	−	a	a	a
32d	+	−	⊦	−	+	−	A	A	a	A	A	−	A	A	a
Bacillus mesentericus															
33	+	+	+	+	⊦	−	a	A	A	a	a	A	a	A	a
34	+	+	+	⊦	⊦	−	a	A	A	a	a	A	a	A	a
35	⊦	+	+	+	+	−	a	A	A	a	a	A	a	A	A
36d	+	⊦	−	⊦	⊦	−	a	A	A	A	a	a	a	A	A
B. polymyxa															
37	+	−	−	+	⊦	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
38	−	−	−	⊦	⊦	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
39	+	⊦	−	+	+	−	AG	AG	AG	AG	AG	AG	AG	AG	Ag
E. solanisapra															
40d	+	−	−	+	+	−	A	Ab	A	Ab	A	A	A	A	A
E. phytophthora															
41d	+	−	+	−	+	−	a	A	A	A	A	−	A	A	a
Escherichia coli															
42d	−	−	+	−	+	−	AG	AG	AG	AG	AG	AG	AG	AG	AG

[a] Cultures 1 to 32 and 40 to 42, inclusive, were Gram-negative; 33 to 39, inclusive, were Gram-positive and spore-formers. All isolates produced short rods and were aerobic

All microorganisms studied were bacteria isolated from infected lenticels on tuber initials and from stems of plants with blackleg symptoms. Single-colony isolations from cultures purified by the dilution-plate method were used in all the identifications and pathogenicity studies reported here. All bacterial isolates and type cultures were maintained on Bacto-nutrient agar or Bacto-nutrient broth. Identification tests were made according to the procedures outlined for pure-culture studies by the Society of American Bacteriologists (6). All bacterial isolates included in the identification studies were first tested for their ability to produce a soft-rot on potato slices in the laboratory. Only those isolates which rotted potato slices were included in the identification studies. The characteristics determined for the various isolates are summarized in table 4.

Gram stain reaction of all isolates was determined by the Hucker modification of the Gram stain. Standard culture 36 was selected as a check for Gram-positive reaction and standard culture 21 for Gram-negative reaction. All isolates were Gram-negative with the exception of 33, 34, 35, 37, 37a, and 38. The Gram-stain reaction was determined on smears prepared from 48-hour Bacto-nutrient agar-slant cultures.

All isolates were stained by the Schaeffer and Fulton modification of the Wirtz method of spore staining. Again, the two cultures used as standards in the Gram-stain reaction were employed as checks. The smears for the spore stains were prepared from 10-day-old Bacto-nutrient agar-slant cultures. Isolates 33, 34, 35, 37, 37a, and 38 were the only ones that produced spores.

Motility was determined in hanging drops prepared from 8-hour Bacto-nutrient broth cultures. All isolates were actively motile except standard culture 42.

Physiological Characteristics

Starch Hydrolysis. The ability of each isolate to hydrolyze starch was determined by means of the starch-agar plate technic. A loop transfer from a broth culture of each isolate was streaked on a plate of Bacto-nutrient

and facultatively anaerobic. All cultures except 42 were actively motile, negative for indole production, and pathogenic on potato slices.

b Each of these types was isolated once, except 1 to 3, inclusive, each of which was isolated three times; 4 to 7, inclusive, and 37 were isolated twice. Isolates identical in reaction to those listed are not included in the table but are referred to in the text as a and b.

c +, Positive for gelatin liquefaction, starch hydrolysis, Methyl Red Test, Voges-Proskauer Test, nitrate reduction, and H_2S production. −, Negative reaction for the above five tests and no visible change in color indicator or gas produced. a, Slight acid production, light pink color. a, Weak acid production, pink to orange color. A, Strong acid production, yellow to clear color. b, 1 to 5 per cent gas by volume in fermentation tube. g, 5 to 10 per cent gas by volume in fermentation tube. G, 10 to 100 per cent gas by volume in fermentation tube.

d Standard cultures 21 and 22 were obtained from Dr. W. H. Burkholder, Cornell University, who considers them typical of *E. carotovora*; 23, 32, 40 and 41 were obtained from the American Type Culture Collection; 39 was obtained from Dr. G. A. Ledingham, Division of Applied Biology, Ottawa, Canada; 36 and 42 from the Department of Bacteriology, University of Minnesota.

agar containing 0.2 per cent soluble starch. All plates were incubated at 26° to 28° C. for 48 hours, after which each plate was flooded with a saturated solution of iodine in 50 per cent ethyl alcohol. Isolates 10, 20, 29, 30, 33, 34, and 35 hydrolyzed starch; the others did not.

Fermentation of Sugars, Alcohols, and Glucosides. All isolates were tested for ability to ferment xylose, dextrose, mannose, sucrose, lactose, and maltose. Glycerol was used to determine the ability of the isolates to ferment an alcohol. Salicin and aesculin were selected to represent the glucosides. Bacto-phenol-red nutrient broth base containing 1 per cent of each of these nine compounds was used for all liquid media. All media for fermentation tests were prepared in Durham tubes, in 5 cc. aliquots, and sterilized for 20 min. at 121° C., except the media containing xylose, which was sterilized with flowing steam for 20 min. on 3 successive days.

The various media were inoculated with a single-loop transfer of a 24-hour broth culture. Each isolate and standard culture was tested in duplicate on each of the 9 media. All cultures for fermentation studies and other physiological tests were incubated at 28° C. The inoculated broths were examined after 24 hours and a daily check was made of each test for 2 weeks. The maximum amount of acid or gas, or both, produced during the 2-week period was taken as the final reading for each culture.

Liquefaction of Gelatin. Liquefaction of gelatin was determined on stab cultures in Bacto-nutrient gelatin incubated at 20° C. Of the 44 isolates studied, 15 liquefied gelatin. The cultures were kept for 28 days; however, all isolates which liquefied the medium did so in 4 to 9 days after inoculation. The type of liquefaction varied from stratiform to infundibuliform, with complete liquefaction in all positive cultures after 28 days. Details are given in table 4.

Reduction of Nitrates. Reduction of nitrates was determined by the sulphanilic acid alpha-naphthylamine test. All isolates with the exception of 25, 27, 29, and 31 reduced nitrates to nitrites. Only one isolate, 28, reduced nitrate with the evolution of gas. Gas production was determined for all cultures in Durham fermentation tubes.

Indole Production. The isolates were tested for the production of indole by the Ehrlich-Bohme method. Duplicate cultures of each isolate were inoculated into Bacto-tryptone broth media and incubated at 28° C. One culture of each isolate was tested for indole production after incubation for 24 hours, and the duplicate, after 48 hours. None of the isolates produced indole. A culture of *Escherichia coli*, 42, that was known to produce indole was included as a check. It gave a positive reaction after 24 and 48 hours.

Production of Hydrogen Sulphide. Hydrogen sulphide production is detected in bacterial cultures by the blackening produced when bacteria are grown in the presence of a metal such as lead, lead sulphide being produced. Only isolates 3, 3a, 3b, 9, and 13 were positive for hydrogen sulphide production.

Methyl Red Test. The methyl red test is used to detect high acidity

produced by certain organisms in the presence of dextrose. This test has been included in comparative studies of soft-rot bacteria by Stanley (29), and Dowson (10, 11). Bacto-M.R.-V.P. medium was used in the methyl red and "Voges-Proskauer Reaction." Of the isolates tested, 3, 3a, 3b, 9, 12, 24, 33, 34, and 35 gave a positive reaction.

"*Voges-Proskauer Reaction.*" This test was made with the same medium as that for the methyl red test. The reactions of the various isolates to this test are in table 4.

As a result of the identification studies four species of bacteria were found associated with lenticel infection; *Erwinia carotovora* (Jones) Holland, *Erwinia aroideae* (Townsend) Holland, *Bacillus mesentericus* Trevisan, and *Bacillus polymyxa* (Prazmowski) Migula. Isolates 1 to 20, inclusive, are believed to be most similar to *Erwinia carotovora* as typified in the reactions of standard cultures 21, 22, and 23. Variation in the physiological tests was recorded; however, it was no greater than that recorded in standard cultures 21, 22, 23, 40, and 41. The variation in the physiological reactions of members of the *E. carotovora* group has been studied by Leach (20). The variation among the isolates here considered to be most similar to *E. carotovora* was no greater than that given by Leach (20) for the group, and that reported by Stanley (28).

Only isolates 10, 15, 17, 19, and 20 liquefied gelatin. If Leach's (20) grouping, which includes *Erwinia solanisapra* (Harrison) Holland, is followed, the variablility in gelatin liquefaction is confirmed. Bergey (2) records surface liquefaction to a depth of 2 mm. after 25 days' incubation for *E. solanisapra*. Where such slight amounts of liquefaction were observed in the present studies, negative reactions were recorded.

Standard culture 41 had a very weak acid reaction in aesculin broth, while all other standard cultures and literature listings for the test show acid production without gas. Those isolates classified here in the *Erwinia carotovora* group gave either a negative aesculin reaction, or both acid and gas positive reaction.

Isolate 9 gave a negative reaction in salicin broth, while all others produced acid and gas. Isolates 3, 8, 9, 13, and 19 produced both acid and gas in glycerol.

In 1904, Townsend (30) described a bacterial soft-rot of calla lily and designated *Erwinia aroideae* as the causal organism. Massey (21) and Wingard (32) reported a soft-rot of tomato fruits caused by *E. aroideae*. Massey (21) concluded from comparative tests of *E. aroideae* and *E. carotovora* that these two organisms, although closely related, should be maintained as separate species. Brierley (4) also included *E. aroideae* in comparative studies of bacteria which caused a rotting of potato tuber tissue under laboratory conditions. He stated, "*Erwinia aroideae* may be found destructive under field conditions also, when a survey of soft rots in the field is undertaken." Stanley (29) and Dowson (10, 11) suggested this organism be classified separately because of its consistent inability to produce gas in the various carbohydrate-containing broth media.

Isolates 24 to 31, inclusive, are believed to be most closely related to *Erwinia aroideae*. The reactions of these 8 isolates to the various tests are recorded in table 4. It is apparent that there is considerable deviation from the standard culture 32. Bergey (2) and Dowson (10, 11) recorded gelatin liquefaction for *E. aroideae*, but isolates 27, 29, and 31 did not liquefy it. Seven of the 8 isolates were negative for both the methyl red and "Voges-Proskauer Reaction," while isolate 24 was positive for both. Four of the 8 isolates apparently had lost the ability to reduce nitrates, while one, 28, reduced nitrates with the production of gas. None of the isolates produced hydrogen sulphide. Bergey (2) has recorded a positive reaction for this test, while a negative test was recorded for the standard culture 32 in these studies. Again, as in comparative studies of the isolates determined to be *E. carotovora*, considerable variation was found among the 8 isolates here assigned to the *E. aroideae* group. It seems most logical to classify the isolates in this group on the basis of their ability to rot potato tubers and their consistent production of acid without gas. Isolate 30 is included in the *E. aroideae* group because it causes rot, and may have produced acid, although in quantities too small to be detected with phenol-red indicator.

Brierley (4) isolated *Bacillus mesentcricus* from a wound rot of potato tubers. Subsequently it rotted healthy potato tubers at temperatures of 20° C. and above in the laboratory. Some spore-forming bacteria of the *Bacillus subtilis* and *Bacillus polymyxa* types can dissolve the middle lamella of parenchymatous tissues (1).

The characters of the 3 isolates of Gram-positive, spore-forming bacilli, 33, 34, and 35, agree with those of standard culture 36 in all tests except the methyl red test, which was positive for all 3 isolates but negative for 36. Isolate 34 appeared as a variant of a culture of isolate 33 and rotted potatoes faster than its parent.

Dowson (12), isolated *Bacillus polymyxa* from stored potato tubers. More recently, Jackson and Henry (18) isolated the same spore-forming bacterium from potato soils in Alberta, Canada, and demonstrated that it could enter through wounds and rot potato tubers at high temperature and humidity.

Three isolates of Gram-positive, spore-forming bacilli, 37, 37a, and 38, gave typical *Bacillus polymyxa* reactions in the various tests. They did not hydrolyze starch, and 38 did not liquefy gelatin. Isolates 37a and 38 were obtained from infected lenticels. Isolate 37 was derived from the base of a potato stem with typical symptoms of blackleg.

DISCUSSION

Potato tuber initials grown in wet soils become infected with several species of bacteria that enter through proliferated lenticels. The higher the soil moisture, the greater was the lenticel proliferation and the number and extent of lenticel infections.

Lenticels are formed on tuber initials early in the development of the

initial. Lenticels near the stem end of the tuber initial may be mature while stomata are still present at the apex of the tuber. Infections occurred most frequently in the stem end two-thirds of the initial, with no infections in the extreme apical region. This observation suggests that lenticels more frequently than stomata are predisposed to infection in wet soils. Those lenticels in the middle third of the initial were more frequently infected than those in the extreme stem end, which further suggests that immature lenticels are more susceptible to infection than mature ones.

In proliferated lenticels, the complementary cells are formed rapidly, are thin walled, non-orderly in arrangement, and have large intercellular spaces. The cork layer, which normally forms beneath the complementary cells, is not present in actively proliferating lenticels in wet soils. Therefore, proliferated lenticels are not protected against entrance of pathogens such as soft-rot bacteria.

The amount of oxygen in wet soils may not be sufficient for suberization of the complementary cells and for cork formation; consequently, proliferated lenticels are further predisposed to infection, as Leach (19) has suggested in the case of blackleg.

Potato tuber initials grown in wet soils where high nitrogen fertilizers had been applied were very susceptible to lenticel infection, probably because of increased rate of complementary cell production, which thus provided less protection to the lenticel.

That the warm, muggy weather that often prevails when soils are wet is conducive to lenticel infection is suggested by the observation that infection of tuber initials was more severe on plants grown in atmospheres with a high relative humidity. The soil temperatures usually encountered under such circumstances would also be favorable to lenticel infection.

The soil environmental conditions in which lenticel infection occurs are also optimum for the growth of the 4 species of bacteria: *Erwinia carotovora*, *Erwinia aroideae*, *Bacillus mesentericus*, and *Bacillus polymyxa*, which are all facultative anaerobes and are known to be more active under partially anaerobic conditions. The anaerobic atmosphere of the rhizosphere was not conducive to the growth of fungi and, in extremely wet soils, prevented the development of Actinomyces. This unnatural balance in the soil microflora may reduce the prevalence of microorganisms that are antibiotic to the soft-rot bacteria. The increased bacterial activity also may be due to a symbiotic relation of the soft-rot bacteria and other bacteria which would not be present in soils of lower moisture content.

SUMMARY

1. Potato tuber initials can be completely rotted at very early stages in their development, especially in wet soils, by at least 4 species of bacteria: *Erwinia carotovora*, *Erwinia aroideae*, *Bacillus mesentericus*, and *Bacillus polymyxa*.

2. The rotting of the initials in wet soils was occasionally so extensive as to suggest that rotting may, in some instances, be responsible for low yields of potatoes.

3. In studies made to determine factors which predispose tuber initials to rot, two soil types were used; sandy loam from the Minnesota Agricultural Experiment Station Farm, St. Paul, and Bridgehampton very fine sandy loam from the Rhode Island Agricultural Experiment Station Farm, Kingston. Rotting was most abundant in both soils when they were saturated to field capacity.

4. Of a total of 30 isolates obtained from infected lenticels on potato tuber initials and potato plant stems grown in University Farm sandy loam, 26 were *Erwinia carotovora* and 3 were *Bacillus polymyxa*. From tuber initials grown in Bridgehampton very fine sandy loam, *Erwinia aroideae* was isolated 8 times, *Erwina carotovora* 4 times, and *Bacillus mesentericus* 3 times. This suggests that the relative prevalence of the soft-rot bacteria may vary in different regions or soils.

5. The development of lenticel infection is retarded by soil temperatures of 18° C. and lower and is accelerated at higher temperatures. All tuber initials on plants grown in saturated soils at 39° C. became infected within 24 hours, whereas the initials on plants grown at 4° and 18° C. required 96 hours for infection.

6. Lenticel infections were more abundant on tuber initials of the Irish Cobbler than on those of the Green Mountain variety in all experiments, but the differences were not statistically significant. The percentage of proliferated lenticels and the extent of proliferation on individual tubers were greater on initials of Cobblers than Green Mountains. There were more lenticels on Cobbler than on Green Mountain tuber initials.

7. In the present study, bacterial infection of tuber initials occurred only through lenticels.

8. Blackleg symptoms were observed on plants grown in saturated soil at temperatures of 21° C. and above, with the optimum for the disease symptoms between 34° and 39° C.

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THE INFLUENCE OF CULTURAL CONDITIONS ON FLAG SMUT OF WHEAT

A . F . E L - H E L A L Y

Flag smut, *Urocystis tritici* Koern., causes an average loss of less than 5 per cent of the wheat crop in Egypt, but under certain conditions it may cause a loss of 50 per cent or more. It is the object of this work to determine the factors which influence the spread and the distribution of the disease in Egypt and to find a cheap method of control.

The disease probably has been present in Egypt for a long time. It attacks the Hindi varieties of wheat, *Triticum vulgare*, which are known to have been grown in Egypt for hundreds of years. The pathogen may also have been introduced to this country with imported Australian grain during the Great War of 1914.

The first record of the disease in Egypt was made by Fahmy (See 7) in 1923. Melchers and others (7) found flag smut at Mansoura in 1928. Jones (5) reported the disease and stated "Ordinarily this disease is very rare, but it appears to be becoming more common and may demand treatment in some cases." Jones and Seif el-Nasr (6) stated that the disease was at its maximum near the center of the Delta: the highest figure of infection ever recorded in a field was 58 per cent, but the average percentage in a normal year was very much less.

It is most severe in lower Egypt (the Delta) and decreases gradually southwards, becoming rare in Upper Egypt. It has been reported in Sinai, but has not yet been seen in the Oases. The intensity of the disease varies from year to year.

METHOD AND MATERIAL

The Hindi D variety of wheat which is susceptible to flag smut disease, was used unless otherwise indicated. The seed was obtained from the Propagation Section of the Ministry of Agriculture. Spores for infection purposes were taken from diseased plants collected especially for this purpose. In infesting the experimental plots, a sample of light soil was infested by mixing it thoroughly with diseased straw and with spores at approximately the rate of 5 gm. of straw and 1 gm. of spores to 1 kilogram of soil. Each experimental plot received, at the proper time, approximately 1 kilogram of this inoculum, which was evenly spread on the surface of the soil and mixed with it to the depth required.

The artificially infested seed was prepared by adding approximately 0.5 gm. spores to 1 kilogram of seed, and then thoroughly mixing them.

The plants of each experiment were kept under observation from the time of sowing to the time of harvest. Readings were taken every fortnight and the total number of the smutted plants per plot were, then, recorded.

The wheat plants of many different plots, each plot being 2×5 meters, were counted and the average number per plot was 750 plants.

This work was carried out in the Field Stations of the Ministry of Agriculture at Dokki and of the Faculty of Agriculture of Farouk 1 University at Damanhour, and in the laboratories of the Plant Pathology Section of the said Ministry during 1940–1941, 1941–1942, and 1942–1943.

FACTORS INFLUENCING DISTRIBUTION OF DISEASE

The spread of the disease in Egypt depends on different cultural and environmental factors which act either together or separately. The main factors which influence the distribution and spread of the disease are: (1) date of sowing; (2) method of sowing; (3) manurial treatment; (4) smut spore load on the seed; (5) number of waterings.

Date of Sowing

Date of sowing plays an important part in the incidence of the disease. Tisdale, Dungan, and Leighty (14) reported that late sowing (after November 1), in Illinois, greatly reduced infection by flag smut. Millikan and Simo (11), in Australia, found that early sowing favored infection. Two experiments were designed in 1940–1941 and in 1941–1942 at Dokki to investigate this point. Artificially infested seed was sown on the following dates: Oct. 1, Oct. 15, Nov. 1, Nov. 15, Dec. 1, and December 15, 1940 and 1941, in artificially infested plots. Each treatment was reported six times. In figure 1, are presented the data obtained and the maximum and the minimum daily air temperatures for each period.

The effect of date of sowing on the incidence of the disease is very pronounced. The plants of the early sowings were almost free from the disease, while those of the late sowings, particularly in 1941, were very badly attacked. The highest infection occurred in the plants of the sowings of Dec. 1. The curves indicate that temperature appears to be the main factor in this case.

Method of Sowing

In Egypt, wheat is sown either by the "Afir" or by the "Herati" method. In the "Afir" method, sowing takes place on dry soil which is irrigated at once, in the "Herati" method sowing occurs on moderately moist soil which is, then, left without irrigation for about one month. These two methods of sowing influence the incidence of certain smut diseases. El-Helaly (2) working on kernel smut of sorghum stated that "Herati" sowing favors considerably the spread of the disease. Jones and Seif el-Nasr (6) working on smut diseases in Egypt found that the crops planted by the "Herati" method consistently bore more flag smut than the "Afir" planting.

In our experimental plots at Dokki, the Herati method of sowing favored infection. In 1940–1941 flag smut infection was 4 to 5 per cent in the four Afir sowings and ranged from 9 to 12 per cent in the four Herati sowings.

The next year, 1941–1942, the smut ranged from 2 to 10 per cent in Afir sowings and from 15 to 34 per cent in the Herati sowings. It seems that the underlying factors in this case may include one or more of the following: water drainage, soil temperature, depth of sowing, and soil moisture.

Drainage of Water of Irrigation at Sowing. Watering which follows sowing in the "Afir" method may remove, during drainage, the spores adhering to the seed coat, leaving the seed almost clean. El-Helaly (2) working on kernel smut of sorghum found that "the soil particles retain the smooth spores and, therefore, do not allow their passage through during
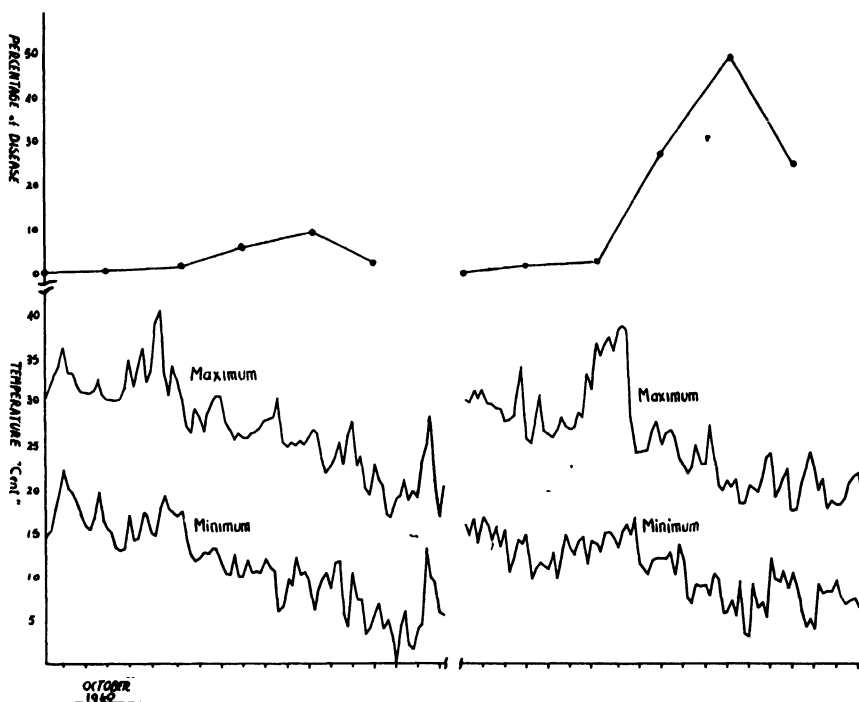
Fig. 1. The percentages of flag smut in plots of Hindi D wheat planted on different dates and the temperatures during the planting season at Dokki, Egypt, in 1940–1941 and 1941–1942.

irrigation." This may be true of the sculptured flag smut spores which may be clustered in spore balls.

Soil Temperature. Soil temperature in the "Herati" method may be more favorable for fungus attack. Jones and Seif el-Nasr (6) stated that the "Afir" plots were a fraction of a degree to two degrees C. cooler than the corresponding "Herati" plots.

Depth of Sowing. In the "Herati" method the seed is broadcast on wet soil and is then ploughed in, giving an average depth of about 8 cms.; in the "Afir" method the seed is broadcast on dry soil and is then covered by means of the zahhafa, a long narrow flat block of wood dragged

over the soil; by this method the seed becomes buried to a depth of about 4 cms.   Miller and Millikan (8), Millikan and Simo (11) and Jones and Seif el-Nasr (6) reported that deep sowing favored infection by *Urocystis tritici*.

In order to ascertain the effect of depth of sowing on the incidence of flag smut disease, experiments were conducted in 1942–1943 at Damanhour using 3 × 7 meters, artificially infested plots.   The plots were drilled with artificially infested wheat grain in strips at a depth of 0.5 cm., 2.5 cms., 5 cms., 7.5 cms., 10 cms., 15 cms., and 20 cms.

The "Herati" method was followed and great care was taken in order that the depths might be as accurate as possible.   Table 1 contains the data obtained from these plots.   The data agree with those of the earlier workers, *i.e.*, the disease increases with the increase in the depth of sowing.

TABLE 1.—*The stand of plants and the percentages of flag smut on three varieties of wheat sown at different depths at Damanhour in 1942–1943*

Depth of sowing, in cm.	Stand[a] and percentage[b] of flag smut in					
	Hindi D		Mabrouk		Hindi 62	
	Stand	Smut	Stand	Smut	Stand	Smut
0.5	15	9	9	8	11	5
2.5	41	28	36	30	42	28
5.0	41	38	37	43	40	33
7.5	42	44	39	57	40	37
10.0	41	44	40	50	40	33
15.0	41	50	33	54	36	41
20.0	37	52	30	66	31	55

[a] The number of plants in a plot, each number being the average of six.
[b] Each percentage is the average for six plots.

It is known that infection occurs when the living fungus comes in contact with recently germinated seedlings.   The occurrence of infection is limited to the period between the time of germination of the seed and the appearance of the seedling above ground.   In deep sowings the coleoptiles remain longer below the surface, growing in damp soil which will, also, keep the infecting fungus alive for a longer time.   This condition will obviously lengthen the period of susceptibility and will, thus, increase the chances of infection.

Soil Moisture at Sowing.   Soil moisture varies according to the two methods of sowing.   With the "Herati" method, the soil is irrigated 8 to 15 days prior to sowing and is left until it is just moist enough for plowing.   Any smut spores that have had soil moisture available for 8–15 days and a natural pre-soaking may put them in condition for germination and infection when the wheat seeds are sown.   With the "Afir" method the soil usually has dried out for 40–50 days prior to sowing and water is first added at sowing time.   There probably is insufficient soil moisture for a pre-soaking of smut spores and the delay in their germination may mean less opportunity for infection of the wheat seedlings.

The work of Noble (13) shows that the spores of flag smut will only germinate after three days' soaking in water and then with difficulty. The addition of small quantities of the tissue of wheat seedlings to spores which had been presoaked in water for several days increased germination. The stimulation by the tissue is greater when the spores have been presoaked for three or more days than when water and fresh tissue are provided simultaneously. Griffiths (4) stated that the spores of flag smut do not germinate readily; but a fairly high percentage of germination occurred by floating the spores on distilled water in a watch glass at a temperature of 18° to 20° C. Germination began in two or three days and proceeded rapidly from the fourth day onwards. Fairly good germination also occurred in distilled juice expressed from wheat seedlings, while in some cases germinating seeds also proved a stimulating medium. Spore germination tests, which were made in our laboratory, confirmed the work of Noble in that the addition of wheat grain or wheat seedling juice to spores which had been presoaked in water for some days greatly increased germination.

This obviously will explain the difference in the incidence of the disease between the moist and the dry soil. The spores in the dry soil will receive water of irrigation just after sowing, i.e., the spores and the seed will be in contact from the beginning. Germination of spores would be poor and chances of infection will, therefore, be slight. The spores in the moist soil, on the other hand, will be under different conditions; for they will have been presoaked for the requisite three days or longer, and will therefore, be stimulated into rapid germination as soon as the wheat grain is planted.

The germination of spores adhering to the grain coat will, also, be poor, as both spores and seed will be present together from the beginning.

Manurial Treatment

The influence of manuring on the severity of flag smut was studied by Forster and Vasey (3), who concluded that applications of lime up to 10 cwt. per acre markedly increased infection, as did applications of farmyard manure; when a mixture of both was applied, infection was particularly severe. Superphosphate appeared to have no effect on the disease. Millikan (9) made sand culture trials and concluded that calcium deficiency inhibited completely the development of flag smut in the susceptible variety Free Gallipoli, nitrogen and potassium deficiencies both tended to decrease the severity of the disease, and phosphorus and magnesium deficiences to increase it. He (10) also stated that a calcium excess of twice the normal dosage significantly increased infection in the Free Gallipoli variety; a four times normal excess had no effect; but severity of attack was increased by combining double calcium with one-fourth magnesium and one-tenth phosphorus. Excess of phosphorus or potassium alone had no effect. With the Ghurka variety no treatment increased susceptibility to any degree comparable with that of susceptible controls grown under · the same conditions.

TABLE 2.—*The percentages of flag smut on Hindi D wheat grown at Dokki in 1941–42 and given different manurial treatments*

Treatment		Average smut infection per plot[a]	
Fertilizer	Amount per feddan	Number of smutted plants	Percentage
Control		299	39.9
Calcium nitrate	200 kilos	396	52.8
Potassium sulphate	Do	324	43.2
Superphosphate	Do	333	44.4
Lime	Do	352	46.9
Farmyard manure (Baladi) .	20 cu. m.	367	48.9

[a] Each figure is an average for the six plots of a treatment.

In order to determine the effect of manurial treatment on the incidence of the disease, an experiment was carried out in 1941–1942 at Dokki, using 2 × 5 meters, artificially infested plots which had been differently manured. Each treatment was repeated six times. Artificially infested grains were used in all cases. The data in table 2 show that infection was severe in all cases, and that all the manures tried increased the incidence of the disease.

Smut Spore Load on the Seed

Millikan and Simo (11) stated that the use of a heavy spore load (1 per cent) for inoculum favored infection by *Urocystis tritici*. Two experiments on this phase of the disease cycle were conducted in 1940–1941 and 1941–1942 at Dokki using artificially infested grain. The smut spore loads added to the grain in 1940–1941 were: none, 0.001 gm., 0.01 gm., 0.1 gm., 1 gm., and 10 gm. In 1941–1942 they were: none, 0.1 gm., 0.5 gm., 1 gm., 5 gm., and 10 gm. per 1000 gm. of grain. Non-infested plots, 2 × 5 meters, were used and each treatment was repeated six times. Table 3 gives the results expressed as averages for six treatments. The data show that the incidence of the disease increases markedly with the increase of smut spore load on the grain.

TABLE 3.—*The flag smut infection in Hindi D wheat[a] infested with varying spore loads*

1940–1941			1941–1942		
Spore load per 1000 gm. of grain	Smutted plants per plot		Spore load per 1000 gm. of grain	Smutted plants per plot	
	Av. No.	Pct.		Av. No.	Pct.
None	2	0.3	None	68	9.1
0.001 gm.	9	1.3	0.1 gm.	252	33.6
0.01 gm.	25	3.3	0.5 gm.	349	46.6
0.1 gm.	57	7.6	1.0 gm.	404	52.5
1.0 gm.	75	10.0	5.0 gm.	580	77.2
10.0 gm.	149	19.9	10.0 gm.	615	82.0

[a] The grain was treated with a surface disinfectant before the flag smut spores were added.

Number of Waterings

Infection takes place when germinating flag smut spores come in contact with recently germinated wheat seedlings. It is, therefore, doubtful whether the amount of water given to the soil during the growing period of the crop influences the incidence of the disease. An experiment in 1941–1942 at Dokki tested the effect of this factor. Artificially infested, 2 × 5 meter plots were used. They were similar except for different numbers of waterings. Each treatment was repeated five times, and the results for each were averaged.

With a single watering at sowing time there were 487 smutted plants in a plot or 64.9 per cent infection; and with two waterings the infection was very similar, 492 smutted plants or 65.6 per cent infected. A slight increase, 71.9 per cent or 539 smutted plants, occurred when three waterings were given the plots. With four waterings, 67.2 per cent were smutted; and with five waterings, 69.3 per cent. The incidence of the disease was very high in all plots, and the number of waterings had no pronounced influence on the amount of infection.

VARIETAL RESISTANCE

It has been noticed during the study of this disease that certain varieties of wheat were susceptible to flag smut, while other varieties were comparatively resistant. An experiment at Dokki in 1941–1942 tested a number of commercial varieties with reference to this point. Artificially infested, 2 × 5 meter plots were used, and each treatment was repeated six times. Artificially infested seed and the "Herati" sowing method were used.

TABLE 4.—*The flag smut infection in eight commercial wheat varieties grown at Dokki in 1941–1942*

Variety of wheat	Smutted plants per plot	Infection
	Number	*Per cent*
Baladi 116	0	0
Baladi "Bouhi"	0	0
Kazouria "Taliani"	0	0
Hindi "Abiad"	201	26.8
Hindi 62 "Dahabi"	242	32.3
Mabrouk "Giza 121"	272	33.6
Hindi D.	325	43.3
Hindi Maarad.	343	45.8

The data in table 4 show that the Hindi and the Mabrouk varieties are very susceptible to flag smut, while the Baladi and the Kazouria varieties are immune.

USE OF FUNGICIDES FOR DISEASE CONTROL

Norwood (12) found that dusting the seed-grain with copper carbonate, Tillatin R, or V.T. 685 was ineffective against flag smut in the case of

heavily inoculated seed-grain. Good control was obtained by steeping the seed-grain in 1.5 per cent copper sulphate solution for three minutes or in a 1:400 formalin solution for ten minutes. Miller and Millikan (8) obtained from 80 to over 90 per cent control with copper carbonate on seed more heavily inoculated than seed normally used by farmers. Dawson (1) dusted wheat seed, heavily inoculated with *Urocystis tritici*, with copper

TABLE 5.—*Flag smut infection in Hindi D wheat grown from seed treated with fungicides and in plots on infested and noninfested soil at Dokki in 1940–41 and 1941–42*

Fungicide	Non-infested soil				Infested soil			
	1940–41		1941–42		1940–41		1941–42	
	Smutted plants per plot		Smutted plants per plot		Smutted plants per plot		Smutted plants per plot	
	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
Control (not treated)	95	12.7	439	58.5	129	17.2	645	86.0
Flowers of sulphur								
5 gr./kilo seed	40	5.3			67	8.9		
10 gm.	53	7.1	205	27.3	39	5.2	205	27.3
Copper carbonate								
5 gm./kilo seed	45	6.0			33	4.4		
10 gm.	40	5.3	240	32.0	38	5.1	355	44.0
Agrosan G[a]								
2.5 gm./kilo seed	28	3.7			44	5.9		
5.0 gm.	21	2.8	218	29.1	29	3.9	297	39.6
10.0 gm.	32	4.3			19	2.5		
Uspulum[a]								
0.5 per cent	36	4.8			44	5.9		
1.0 per cent	59	7.9			56	7.5		
2.0 per cent	32	4.3			33	4.4		
Germisan[a]								
0.5 per cent	69	7.9	176	23.5	41	5.5	323	43.1
1.0 per cent	39	5.2			50	6.7		
2.0 per cent	56	7.5			40	5.3		
Copper sulphate								
0.5 per cent	28	3.7	170	22.7	26	3.5	177	23.6
1.0 per cent	31	4.1			33	4.4		
2.0 per cent	22	2.9			26	3.5		
Lime-Sulphur								
0.5 per cent	201	26.8			117	15.7		
1.0 per cent	114	15.2	274	36.5	194	22.9	316	42.1
2.0 per cent	55	7.3			48	6.4		
Bordeaux mixture								
0.25 per cent	85	11.3			101	13.5		
0.5 per cent	45	6.0			87	11.6		
1.0 per cent	48	6.4	287	38.3	45	6.0	335	44.7
Formalin								
0.5 per cent	21	2.8	124	16.5	19	2.5	132	17.6

[a] Mercury is the toxic ingredient of the fungicide.

carbonate (2 oz. per bushel) or dipped the seed in 1.5 per cent solution of copper sulphate and then in lime-water and got 4.33 per cent infection in the dry treatment, 7.3 per cent in the wet treatment, and 21.6 per cent infection in the untreated controls. The copper sulphate treatment impaired the vitality of the seed, and it is not recommended.

Two experiments were designed in 1940–1941 and 1941–1942 at Dokki

to determine the effect of certain fungicides in controlling the disease in Egypt. Artificially infested and noninfested plots, 2×5 meters, and artificially infested seed were used. Each treatment was repeated four times.

The following conclusions may be deduced from the data in table 5.

The two lower concentrations of lime-sulphur definitely increase susceptibility to disease.

The lower concentration of Bordeaux mixture is not satisfactory.

Flowers of sulphur, formalin, copper carbonate, Agrosan G, Upsulun, Germisan, copper sulphate, and the higher concentrations of lime-sulphur and Bordeaux mixture gave partial control.

There was more disease in plants on infested soil than in plants on noninfested soil.

We also determined the effect of the different fungicides on germination of the treated seed and growth of the seedlings. Formalin and the two higher concentrations of Germisan and copper sulphate had a bad effect on seed germination and growth. The other fungicides had no harmful effect on the plants.

SUMMARY

Flag smut is distributed throughout Egypt. It reaches its maximum in Lower Egypt (the Delta) and, then, decreases gradually southwards until it becomes rare in Upper Egypt. The intensity of the disease varies from year to year.

The loss caused by the disease in Egypt is small; it does not, usually, exceed 5 per cent, but under certain conditions it may become so destructive as to cause a loss of more than 50 per cent.

The plants of the early sowings are almost free from the disease; while those of the late sowings are badly attacked. Temperature appears to be the main factor responsible.

"Herati" sowing favors considerably the attack. Both depth of sowing and soil moisture at sowing play an important part in this connection. The seed is, usually, sown deeper in the "Herati" method than in the "Afir" method and the spores in the "Herati" are usually subjected to presoaking for a period of 8–15 days before sowing, while those in the "Afir" remain dry until sowing time, when the first irrigation is used. The disease increases with the increase in depth of sowing and with the presoaking of the spores before sowing.

No promising control was evident in manurial treatment of the soil. All manures tried increased the incidence of the disease.

The amount of water given to the soil during the growing period of the crop has no influence on the incidence of the disease.

The Hindi and the Mabrouk varieties of wheat are very susceptible to flag smut; while the Baladi and the Kazouria varieties are immune.

Partial control of the disease with no harmful effect on germination of the treated seed and growth of the plants is given by the use of 0.5 per

cent Germisan, 0.5 per cent Upsulun, flowers of sulphur, copper carbonate, and Agrosan G. "Afir" sowing, clean seed and soil, and early sowing are highly recommended. Cost of treatment with any of the recommended fungicides is very small and negligible.

The incidence of the disease increases with the increase of smut spore load on the seed.

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STEM-END ROT OF STRAWBERRIES

Const. J. Alexopoulos and Donald Cation

INTRODUCTION

The study of various fruit rots of strawberries has commanded the attention of a large number of investigators wherever strawberries are grown and particularly in the United States. A number of organisms have been connected with the spoilage of the fruit in the field, in transit, and on the market. The most important of these, it is generally agreed, are various species of *Botrytis* of the *cinerea* type causing gray rot, and *Rhizopus nigricans* causing leak. Both diseases are considered serious in the United States (6, 19, 26, 27, 28), and have also been reported from Canada (4, 9) and Australia (1, 22), while damage from *Botrytis* is important commercially also in Argentina (15), Germany (12), Japan (13), New Zealand (5), Norway (14), and Russia (24). A more thorough search of the literature would without doubt reveal many more reported instances of these organisms attacking strawberries in other parts of the world.

In addition to these two well known rots, tan rot (*Pezizella lythri*), hard rot (*Rhizoctonia* sp.), and leather rot (*Phytophthora cactorum*) are common, especially in the United States (6, 10, 20, 21, 28), though not so prevalent as the first two previously mentioned. *Mycosphaerella fragariae*, the well known cause of leaf spot, was found to cause "black seed" disease of strawberries, particularly in Maryland and North Carolina according to Demaree and Wilcox (11) who first described this disease. - A *Septoria*, however, presumably the conidial stage of *M. fragariae*, had been previously reported from England by Ogilvie in 1932 (17) as the cause of a hard rot of strawberries. Finally, *Sclerotinia sclerotiorum* is listed on strawberry fruits in the United States (21), *Didymella lycopersici* has been reported (23) as attacking flowers and fruits as well as leaves and roots of strawberries in England, and *Sclerotium rolfsii* to cause a soft rot in Florida (16). Stevens and Peterson (25) described a fruit rot of strawberry which they found first in Louisiana in 1914, and which, they state, was later found to be very common on berries in the market at Urbana, Illinois. They described the causal organism, a pycnidial fungus, as *Sphaeronemella fragariae*. These seem to be the only organisms which have been associated with fruit rots of strawberry.

FIELD AND LABORATORY OBSERVATIONS

In connection with some spraying experiments which had been started by the junior author, the strawberry plantation of Mr. Max Smith, near Lawrence, Michigan, was visited on July 2, 1947. A number of berries of the Robinson variety with disease symptoms were collected outside the experimental plots. Each was wrapped in newspaper separately, and placed with

others in a paper sack. Upon return to the laboratory the same evening, the sack was placed in the refrigerator.

On July 3, several of the berries were examined under the binocular dissecting microscope in an attempt to diagnose the various rots symptomatologically. Such diagnosis was verified when there was any doubt, by isolations from the diseased tissues. As the examination of the berries continued, it was noted that a comparatively large number had a brown discoloration accompanying a general dry rot which seemed to begin at the stem end. Further examination revealed the pycnidia of a fungus forming on the berries below the calyx. These pycnidia contained spores closely resembling those of *Dendrophoma obscurans*, the cause of leaf blight.

Up to July 7, fifty berries in all had been individually examined. Of these, 33 bore pycnidia. The prevalence of a fruit rot presumably due to *Dendrophoma obscurans* was interesting, as the fungus, well known as the cause of leaf blight in many localities in the United States as well as in Argentina (8), and new South Wales (3), has never been connected with a fruit rot as far as the writers have been able to determine. Pycnidia of *Dendrophoma* were also frequently observed on living or dead calyces and on the dead peduncles of the berries under scrutiny. It appeared as though the infection was started in the field on the peduncles and entered the berry after killing the stem.

The prevalence of *Dendrophoma* on the berries was emphasized when the examination of all the berries, 1215 in number, representing two pickings from six small experimental spray plots on Mr. Smith's plantation was completed. The first picking took place on July 4, 1947, and the second on July 7. These two pickings will be referred to as Lot I and Lot II, respectively.

The usual well known rots were represented in these two lots. In addition, a "stem-end rot" and a "soft rot" neither of which showed any external signs of a fungus, were found, as well as the "Dendrophoma rot" characterized by the presence of the pycnidia. Because it was suspected that all three of these may be stages of the same disease, all berries were returned to their boxes after examination, each group separated by a layer of newspaper, and kept in the laboratory for examination at a later date.

The initial examination of the fruit was completed on July 9 for Lot I, and July 10, for Lot II. On July 11 and 12 respectively, the two lots of berries were reexamined. As was suspected, it was found that a large number of the fruits previously classified in the groups "stem-end rot" and "soft rot," had developed pycnidia of *Dendrophoma*.

A final examination of the berries affected with "stem-end rot" and "soft rot" on July 11 and 12, was made on July 14 (Table 1).

Thus, 618 strawberry fruits out of 1215 picked and individually examined, developed pycnidia of *Dendrophoma*. This represents 50.8 per cent of all berries picked, and 76.3 per cent of all diseased berries in the two lots. It is not improbable that the 48 berries which were still classified in the

"stem-end rot" and "soft rot" categories at the end of the observations, would eventually develop pycnidia. The seriousness of the disease in these plots is evident from the above figures.

TABLE 1.—*Incidence of fruit rot in two lots of Robinson strawberries picked July 4 and 7, 1947, respectively, near Lawrence, Michigan*

Condition of berries	Lot I			Lot II		
	July 9	July 11	July 14	July 10	July 12	July 14
Sound	239	239	239	171	171	171
Pycnidia of *Dendrophoma*	87	272	289	159	292	329
"Stem-end rot"	161	32	16	63	11	3
"Soft rot"	69	15	14	125	44	15
Gray rot (*Botrytis* sp.)	36	36	36	30	30	30
Tan rot (*Pezizella*)	14	14	14	52	52	52
Leather rot (*Phytophthora*)	5	5	5	1	1	1
Leak (*Rhizopus*)	1	1	1	0	0	0
Blue mold (*Penicillium*)	1	1	1	1	1	1
Total	613	615[a]	615[a]	602	602	602

[a] Two berries having both *Dendrophoma* pycnidia and *Botrytis* conidiophores, are included twice.

THE DISEASE

Stem-end rot of strawberries appears to invade the fruit from infected peduncles. The most typical symptom is a brownish discoloration of the upper part of the berry, at first under the calyx, accompanied by a softening of the tissues. The softening of the tissues eventually spreads to the whole fruit giving rise to a general soft rot. The berry gradually begins to shrivel, the portion beneath the calyx drying and often developing into a depression. Pycnidia of *Dendrophoma obscurans* develop under the epidermis, become erumpent and exude large quantities of pycnidiospores from the ostiole. The pycnidia are at first hidden from view by the calyx lobes but later develop on other parts of the berry as well. The fruit eventually mummifies.

IDENTIFICATION OF THE ORGANISM

Upon tentative identification of the organism as *Dendrophoma obscurans*, strawberry leaves of the Dunlap variety with typical symptoms of *Dendrophoma* leaf blight were collected at East Lansing, Mich., brought to the laboratory, and placed with their petioles in water under a bell jar. A few days later they were examined for pycnidia. Many pycnidia were found which answered the description of *D. obscurans*. Conidiophores and spores from leaves, berries, calyces, and peduncles, corresponded closely; pycnidia on leaves, however, tended to be small and dark. Pure cultures obtained by transferring pycnidiospores from berries, leaves, and calyx lobes, to maltose agar, and from plantings of diseased berry tissue taken from the inside of berries as far away as possible from the location of the pycnidia, were in all ways similar. All produced pycnidia and pycnidiospores identical with those on various parts of the host.

THE PERITHECIAL STAGE

Of various cultures on maltose agar inoculated on July 3, from the berries picked on the previous day, one labelled S6 proved particularly interesting. Diseased tissue from a berry on which no external signs of a fungus were as yet evident, but which exhibited symptoms of stem-end rot, had been planted on five places in a Petri dish containing maltose agar. Of the five colonies developed, one gave rise to typical sporodochia of *Pezizella lythri*, while the other four eventually developed round, black fruiting bodies with long beaks. On July 17, one of these structures gave forth, when crushed, a large number of immature asci. Pycnidia interspersed among the perithecia contained
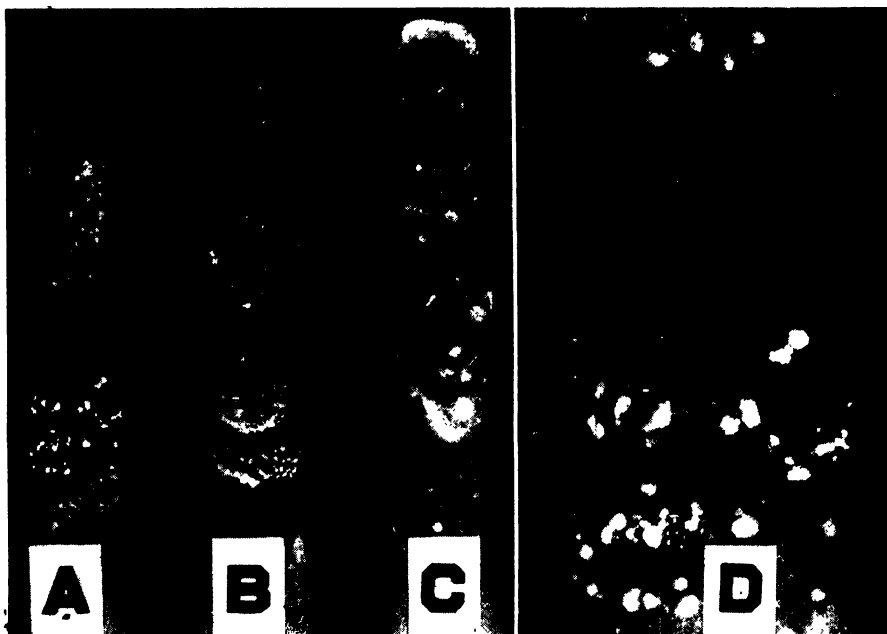
FIG. 1. Three single pycnidiospore cultures (A, B, and C) of *Dendrophoma obscurans*, on corn meal agar, isolated from (A) strawberry leaf with typical symptoms of leaf blight, (B) infected strawberry calyx, and (C) infected strawberry fruit. Note band of perithecia among the pycnidia in (A) and in (D) which is an enlarged view of (A). Subsequent transfers on corn meal agar from (B) and (C) also produced an abundance of perithecia. Photographs by F. C. Strong.

pycnidiospores typical of *Dendrophoma obscurans*. The connection between the two stages appeared probable. Six other isolations from different berries yielded pure cultures of *D. obscurans*, with an abundance of pycnidia, but no other stage except a single black fruiting body with two long beaks, similar to the perithecia in culture S6, buried in the agar of culture S15.

On the same day, beaked structures similar to those found on agar, were discovered on the calyx of a berry which had been kept in a moist chamber for approximately two weeks. Under the binocular dissecting microscope, black, bristle-like structures, the beaks of perithecia buried in the calyx

tissue, were seen protruding through the epidermis. The globose base was easily dissected out of the host tissue and when crushed in water under a cover glass gave forth an oil exudate together with some cellular matter indicating the beginning of ascus formation. On the following day, more perithecia were found on the calyces and peduncles of berries which had been kept in the refrigerator, wrapped in paper since July 2. Definite asci with immature spores issued from one that was crushed. Later in the season (Sept. 9), fully mature ascospores were found in perithecia that had developed on the strawberries in a moist chamber. All berries, on the calyces of which perithecia were found, were heavily infected with *Dendrophoma obscurans,* as evidenced by the abundant pycnidia at the stem end. Pycnidia were also invariably associated with the perithecia on the calyces and the peduncles.
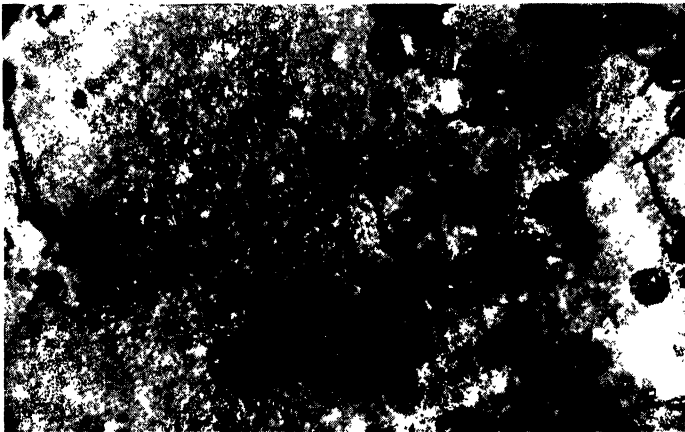
FIG. 2. Perithecia of *Gnomonia* sp., the perfect stage of *Dendrophoma obscurans,* on corn meal agar. × 69. Photograph by F. C. Strong.

Anderson (2) who searched leaves diligently for the perfect stage of *Dendrophoma obscurans* from September to May, failed to find it. He suggests that since the conidia are able to overwinter, a perfect stage would not be necessary for the survival of the fungus. The writers have not searched for perithecia on leaves. The only perithecia found in the field were collected on July 21 on the calyces and peduncles of two small, mummified berries in a Dunlap strawberry patch in East Lansing.

Several single pycnidiospores were now isolated from leaves, calyx lobes, and berries, in an attempt to obtain proof of the connection between *Dendrophoma obscurans* and the new ascomycete. The colonies resulting from all of these were in all respects similar. On July 24, transfers were made to corn meal agar slants and stored away. When they were examined on September 10, both perithecia and pycnidia had been formed abundantly by two cultures, DL2.2 and DL4.2, both derived from single pycnidiospores isolated from leaf tissue. The pycnidia were scattered over the slant, but

the perithecia were crowded together forming a black band at the base of the slant (Fig. 1, 2).[1]   All other slants had produced pycnidia but no perithecia. Subsequent transfers from cultures originally isolated as single pycnidiospores from calyx, berry, and leaf, have produced an abundance of mature perithecia on corn meal agar in Petri dishes (Fig. 2).   No perithecia have ever been found on the few cultures on potato-dextrose, oat, plain (nutrient), or maltose agar, with the exception of the two plates of S6 and S15 on maltose, previously mentioned.   This experience explains why Anderson (2), who worked with oat agar, failed to produce the perfect stage in culture. Experiments are in progress to determine the factors affecting perithecial production.

In old Petri-dish cultures containing perithecia, the ascospores are released from the perithecium through the long, beaked ostiole, in a droplet of liquid which either remains as a glistening droplet at the tip, or rolls part way down, often forming a collar around the neck.   Pure cultures were easily obtained by touching a sterile platinum needle to the droplet containing ascospores, and transferring them on agar.   Such cultures gave an abundance of pycnidia of *Dendrophoma obscurans* on potato-dextrose, and oat agar, and the colonies were indistinguishable from those derived from pycnidiospores planted at the same time on the same types of media.

The cultural experiments, described above, are offered as evidence of the connection between *Dendrophoma obscurans,* and the perithecial stage herein discussed.

CLASSIFICATION OF THE PERFECT STAGE

The characters of the perithecial stage, place the organism in the genus *Gnomonia* of the Gnomoniaceae in the Sphaeriales.   The black, globose perithecia, are deeply buried in the host tissues from which their long necks project; the walls are more or less leathery; there is no stroma.   Asci are formed, but when the spores mature, the ascus wall deliquesces and the ascospores are released in a droplet of liquid through the ostiole.   When perithecia containing mature or very nearly mature ascospores are crushed, the spores released cling together in the same position in which they were formed in the ascus, but no ascus wall can be demonstrated.   Application of pressure on the cover glass will cause the ascospores to separate.   They do so with difficulty, however, because they are held together in a viscid matrix.

The ascospores are one-celled at first, but a septum develops by the time they are released from the perithecium, dividing them into two unequal cells. The spores are curved.   They are characterized by five to seven rather large refractive droplets, the presence of which makes it difficult to distinguish the septum.   Most spores examined had three of these droplets in each cell.   Old spores become highly vacuolated.

Perithecia from corn meal agar measure 236–545 μ in diameter, the

[1] The writers are indebted to Professor F. C. Strong of the Department of Botany and Plant Pathology, Michigan State College, for the photographic work in connection with this investigation.

length of the neck varying from 600 μ to 1455 μ. Ascospores from the same source, measured 7–11.5 × 2–3 μ. The species of *Gnomonia* to which the fungus belongs has not been determined as yet. Studies of the fungus in culture, as well as host inoculation experiments are in progress and will be reported as soon as sufficient data are on hand.

<div align="center">DISCUSSION</div>

The perithecial stage of *Dendrophoma obscurans*, as reported herein, appears to be very similar to that of *Zythia fragariae*, the cause of strawberry leaf blotch found in England by Wormald (29). Strawberry leaf blotch was described in 1941 by Wormald and Montgomery (31, 32) and was at that time ascribed to *Phyllosticta grandimaculans* Bubak and Krieger (7). In spite of the great resemblance, neither Bubak nor Petch (18) who identified Wormald's fungus as *Zythia fragariae* Laibach (*Phyllosticta grandimaculans* B & K), mentioned any resemblance to *Dendrophoma obscurans* (E & E) Anderson. Nor does Wormald mention the American fungus which he obviously considers distinct. With similar perfect stages discovered for the two organisms, a comparison of material from England and America should prove interesting. It is of further interest to note that *Sphaeronemella fragariae* St. et Pet., found on strawberries by Stevens and Peterson (25) is also a pycnidial fungus and that it has many characters in common with both *Zythia fragariae* and *Dendrophoma obscurans*. According to the description, it differs from these two chiefly in the size of the pycnidia—an admittedly variable character—and in the fact that its conidiophores are described as simple. Simple as well as branched conidiophores, however, are described for *Zythia fragariae* by Wormald, and are common with our fungus. Although Stevens and Peterson grew their organism on corn meal agar, no perithecial stage was reported.

In his discussion of Dendrophoma leaf blight of strawberry, Anderson (2) states: "While the disease causes serious loss of functioning leaf tissues, it is not considered of sufficient economic importance to warrant spraying or other expensive control measures." The same opinion is expressed by Demaree (10). If further observations show that stem-end rot of strawberries is as severe elsewhere as it was determined to be in the small experimental plots near Lawrence, Mich., and as it was observed on Dunlap in East Lansing in 1947, it may be very much worthwhile economically to devise control measures for this disease.

<div align="center">SUMMARY</div>

A disease described as stem-end rot of strawberries, presumably caused by *Dendrophoma obscurans*, the cause of strawberry leaf blight, was found to cause severe damage to strawberry fruits in some plots under observation near Lawrence, Michigan, in 1947. As much as 50.8 per cent of all fruits picked from six small experimental plots were affected. The organism isolated from fruits, calyces, and peduncles of diseased strawberries yielded

colonies and fruiting bodies on agar indistinguishable from those produced by isolations of *Dendrophoma* from typical leaf blight lesions.

A perithecial stage assigned to *Gnomonia* was discovered on agar and on calyces and peduncles of strawberries in association with the pycnidia of *Dendrophoma obscurans*. Pycnidiospore plantings on agar yielded perithecia, and ascospore sowings yielded pycnidia. The species to which the perfect stage belongs has not been determined as yet.

The similarity between *Dendrophoma obscurans* and its perfect stage on the one hand, and *Zythia fragariae* and its *Gnomonia* perfect stage in England on the other, are briefly discussed.

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TWO INTERESTING LEAF SPOTS OF FIG

E . C . T I M S A N D L . S . O L I V E

INTRODUCTION

In September, 1939, a very conspicuous leaf spot was observed on a fig tree (*Ficus carica*), Celeste variety, in southern Louisiana. During the summer and fall of the following year, it was observed in two other localities. In 1942, the same disease was found in several widely scattered locations in the State. The first infections were found late in the season and were apparently of little importance, but in 1942, a small group of heavily infected fig trees bearing immature fruit was found near Baton Rouge in early July. These trees were partially defoliated and quite unsightly in appearance. Since this leaf spot was apparently different from any previously described on fig, it was investigated further. The disease was designated as "Cephalosporium leaf spot" (6).

About the same time that the Cephalosporium spot was found, another very similar leaf spot was observed on fig trees in the same localities. The spots were so similar in general appearance that at first they were considered to be due to the same fungus. Later observations indicated, however, that the second spot was somewhat different from the one caused by *Cephalosporium*. The fungi associated with the two leaf spots were entirely different.

The two leaf spots developed at about the same time, usually in July or early August, often on the same tree and occasionally on the same leaves. The Cephalosporium spots could be distinguished by the presence of cottony white tufts of a fungus on the lower surface, while the lower surface of the spots of the second disease had a smoky, brownish gray fungus growth in the center surrounded by a ring of white mycelium.

Apparently neither of these leaf spots on fig had been previously described. A "zoned" leaf spot of coffee associated with *Cephalosporium* sp. was reported by Fawcett (2) from Puerto Rico in 1915. In its early stages the spot illustrated in his paper is similar in appearance to the one on fig. Fawcett isolated the causal fungus and reproduced the disease with pure cultures of the *Cephalosporium*. Later Abbott (1) reported a zonal leaf spot of coffee in Peru which he attributed to *Cephalosporium*. Neither of these workers gave a specific name to the fungus.

The two new leaf-spots of fig are described in this paper and their causal organisms are named.

THE CEPHALOSPORIUM SPOT
(Fig. 1, A and B; Fig. 2, A–D)

Symptoms

The small, circular, brown spots first appear on the upper surface of the

leaves. As they enlarge a series of irregular concentric rings is formed, light brown bands of dead tissue alternating with darker brown rings. The spots may enlarge to form as many as a dozen rings. The centers of the spots often fall out, giving the leaves a ragged appearance. Soon after the spots appear, the characteristic small fluffy, cottony, white tufts of mycelium develop on the lower surface in large numbers. These cottony tufts are made up of cords of hyphae that have surrounded the leaf hairs. The small cephalosporia (heads) with numerous small, hyaline, single-celled conidia are produced on slender phialides projecting from the cords of hyphae (Fig. 2, B–D). The white tufts persist on the lower surface of the spots until the dead areas collapse and fall out. A typical infected leaf is shown in figure 1, A and B.

Technical description

Cephalosporium fici sp. nov.

Maculis manifestis, 1–6 cm. diametro, in superficie superiori zonatis, auctione subalba plumosaque in superficie inferiori tectis. Hyphis per partes superiores pilorum folii se glomerantibus ut funes hypharum fiant qui phialides in ipsis vel in ramis brevibus lateralibusque efficiant. Phialidibus gracilibus, septum in basibus frequenter atque septum unum vel duo supra habentibus, 2.4–4.0 × 34–99 μ. Conidiis singulis in apicibus phialidum effectis, se in globos 6.9–12.2 μ diametro glomerantibus; conidiis ovatis usque ad forman cylindricam, rectis vel subcurvatis solam cellulam hyalinam, 1.9–4.5 × 3.8–10.4 μ mediis inter maximum minimumque 3 × 6 μ, habentibus.

Parasiticum in foliis, *Fici caricae*.

Spots very conspicuous, 1–6 cm. in diameter, distinctly zonate on the upper surface of the leaf, with light brown bands of dead tissue alternating with more narrow and darker brown rings, lower surface covered with a whitish fuzzy growth of the fungus and densely stippled with white dots where the sporulating cords of hyphae have climbed the leaf hairs; dead parts of the leaf spots frequently falling out.

Internal mycelium intercellular, hyaline, giving rise to the external mycelium through the stomates, the external mycelium hyaline, hyphae climbing the leaf hairs and massing together along the upper portions of the trichomes to produce compact cords of hyphae, measuring 18–55 × 250–400 μ, giving rise to phialides directly or from short lateral branches. Phialides slender, tapering gradually towards the apex, each phialide frequently cut off by a cross wall at its base, 1–2-septate or non-septate above, measuring 2.4–4.0 × 34–99 μ. Conidia produced singly at the tips of the phialides but aggregating into globose masses measuring 6.9–12.2 μ in diameter, conidia oval to cylindrical, straight to slightly curved, 1-celled, hyaline, measuring 1.9–4.5 × 3.8–10.4 μ, averaging 3 × 6 μ. Parastic on leaves of *Ficus carica;* E. C. Tims, Schriever, Louisiana; September 17, 1947.
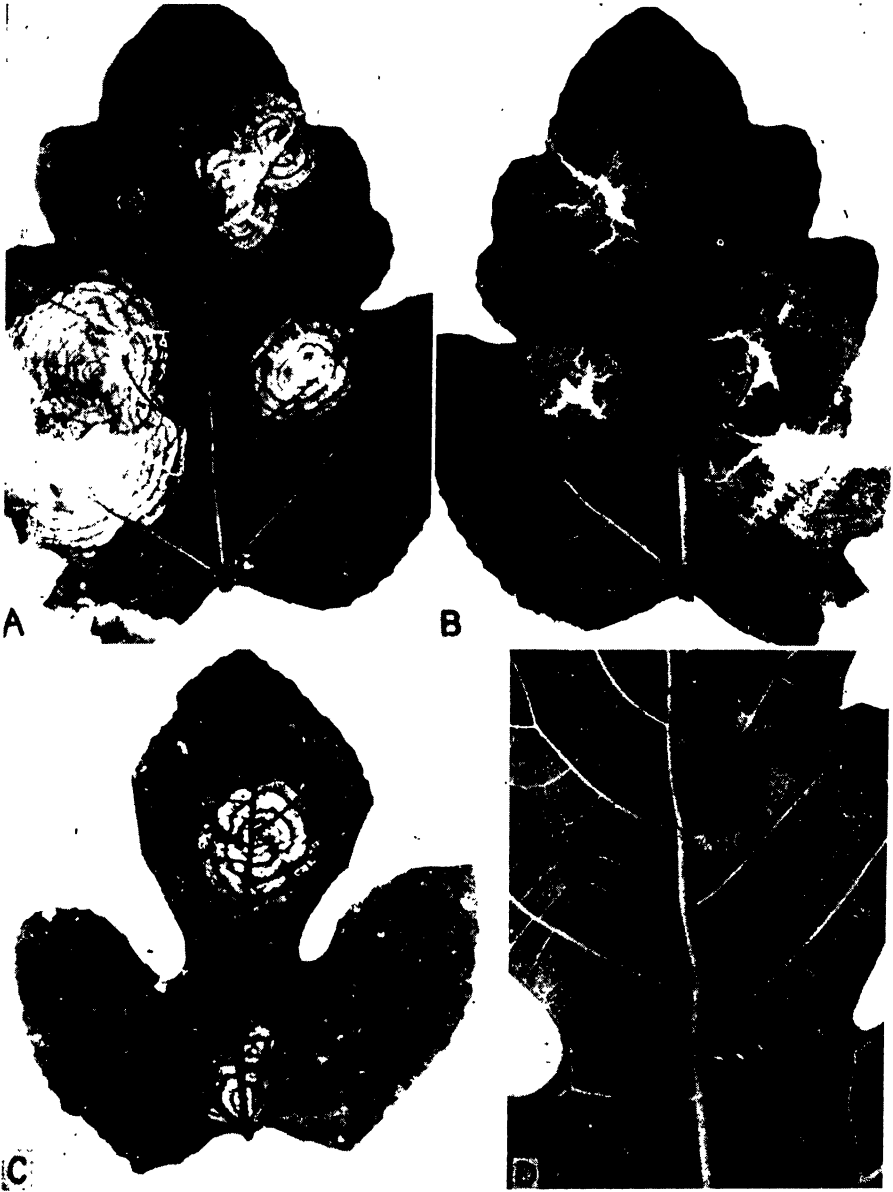
FIG. 1. The two leaf spots of fig. A and B, *Cephalosporium fici;* C and D, *Ormathodium fici.* A, upper surface; B, lower surface of the same leaf, natural infection. C, upper surface of the Ormathodium spot, natural infection; D, a view of the lower surface of an artificially infected leaf.

Cultural characters

Cephalosporum fici was isolated from typical lesions on fig leaves, both by tissue transfers and spore dilutions. The fungus grew well and produced numerous spores on several culture media. On Czapek's agar

growth was white, becoming slightly pink as the cultures aged. Dense, raised, cottony masses of mycelium developed on this agar. On water agar growth was appressed, with practically no aerial mycelium. The fungus produced characteristic large ropes of hyphae on string bean agar. One of the smaller cords of hyphae with whorls of phialides is shown in figure 2,.D. Conidia were borne in heads on slender tapered phialides (Fig. 2, A). They were similar in size and shape to the conidia taken directly from diseased fig leaves.

Taxonomy

When the fig leaf spot was first observed (6), the causal fungus was thought to be *Cephalosporium acremonium* (Corda). Later examination, however, showed that the fig fungus differed in some respects, notably in spore size, from the type *C. acremonium* associated with the black-bundle disease of corn. Reddy and Holbert (4) made a study of *C. acremonium* in which they discussed the literature rather thoroughly. The spore measurements and illustrations of spores of *C. acremonium* as given by these writers are distinctly different from those of the fig fungus. The fig *Cephalosporium* resembles *C. carpogenum* Ruehle as described by him (5), but again there are important differences in the size of conidia as well as the size of phialides.

The *Cephalosporium* causing the leaf-spot of fig is apparently different from any previously described species. For this reason and also in view of the fact that no species of *Cephalosporium* or morphologically similar fungi have been previously reported on *Ficus carica*, the writers consider it best to regard this as a new species.

Pathogenicity

Mature fig leaves on orchard trees were successfully inoculated in two ways during the summer months: (1) by spraying them with spore suspensions, and (2) by placing small pieces of a bean pod culture of the fungus on the lower leaf surface. After the latter type of inoculation, the leaves were wrapped in moist paper and covered with paper bags to hold in the moisture for 24 to 48 hours. During periods of warm, wet weather in July or August, symptoms appeared on leaves inoculated by each of the above-mentioned methods within 4–6 days, and the spots enlarged rapidly, reaching a diameter of 3–5 cm. within two weeks. The centers of such spots often fell out, leaving ragged, irregularly shaped holes in the leaves similar to those observed in naturally infected leaves.

Apparently most infection takes place through the lower surface of the leaves, although occasionally infection was produced by inoculating the upper surface. Within a week after infection, the small white fruiting structures of the fungus were observed on the lower surface of the leaf. Pure cultures of the *Cephalosporium* were reisolated by making spore dilutions from such material.

Primary inoculum probably comes from old infected leaves on the

ground, since the first infected leaves are invariably those nearest the ground.   Early in the summer of 1940 some inoculations were made with *Cephalosporium fici* on a small isolated group of fig trees several miles away from any known Cephalosporium-infected trees.   Later in the season the fungus spread to other trees nearby.   Natural infection occurred on all the trees in the group the following summer.

THE ORMATHODIUM SPOT
(Fig. 1, C and D; Fig. 2, E–J)

Symptoms

The spots vary from 1 to 8 cm. in diameter and are roughly circular. They first appear as small round, dark brown lesions that gradually enlarge, forming definite concentric rings on the upper surface of the leaf (Fig. 1, C).   The older portions of the spots fade out and soon become papery white.   There may be from 2 to as many as 12 concentric rings formed in a single spot, the older rings in the center becoming almost perfectly white with age.   The outer ring may vary from light brown to a very dark brown.   The dark outer rings usually appear when the spots are young and growing rapidly.   In the old spots the white papery centers often fall out and leave ragged holes in the leaves.

The lower surface of the spots also has a characteristic appearance (Fig. 1, D).   The centers are usually brown in the younger spots, with distinct whitish borders where the fungus mycelium is developing on the surface. The brown center with the white mycelium around the border is characteristic of this leaf spot.   As the spots become older the smoky-grayish brown masses of conidia and conidiophores of the causal fungus usually appear in the centers.   Microscopic observation reveals the presence of numerous septate and much-branched conidiophores bearing curved, septate conidia (Fig. 2, E–G).   This development of conidia does not always occur, but when moisture is plentiful they are usually found.   Some fig trees have been observed throughout an entire season with numerous spots on the lower leaves, but few conidia were produced on any of them.

The spots seldom appear on the fig trees in south Louisiana before early July and are usually not conspicuous until later in the summer.   They generally show up on the very lowest leaves and seldom appear on the upper half of the tree.   The disease was very conspicuous on some of the affected fig trees in late August.   Practically all the lower leaves had from one to several of the large spots on them.

Technical description

Ormathodium fici sp. nov.

Maculis magnis, brunneis, aliquanticulum concentrice zonatis.   Mycelio intra folium inter cellulas sito, hyalino; mycelio externo subbrunneo, ?–4.5 μ diametro.   Conidiophoris subbrunneis, ramosis, multiseptatis, nodos manifestos habentibus, in quibus conidia feruntur.   Illa 3–4 × 135–240 μ
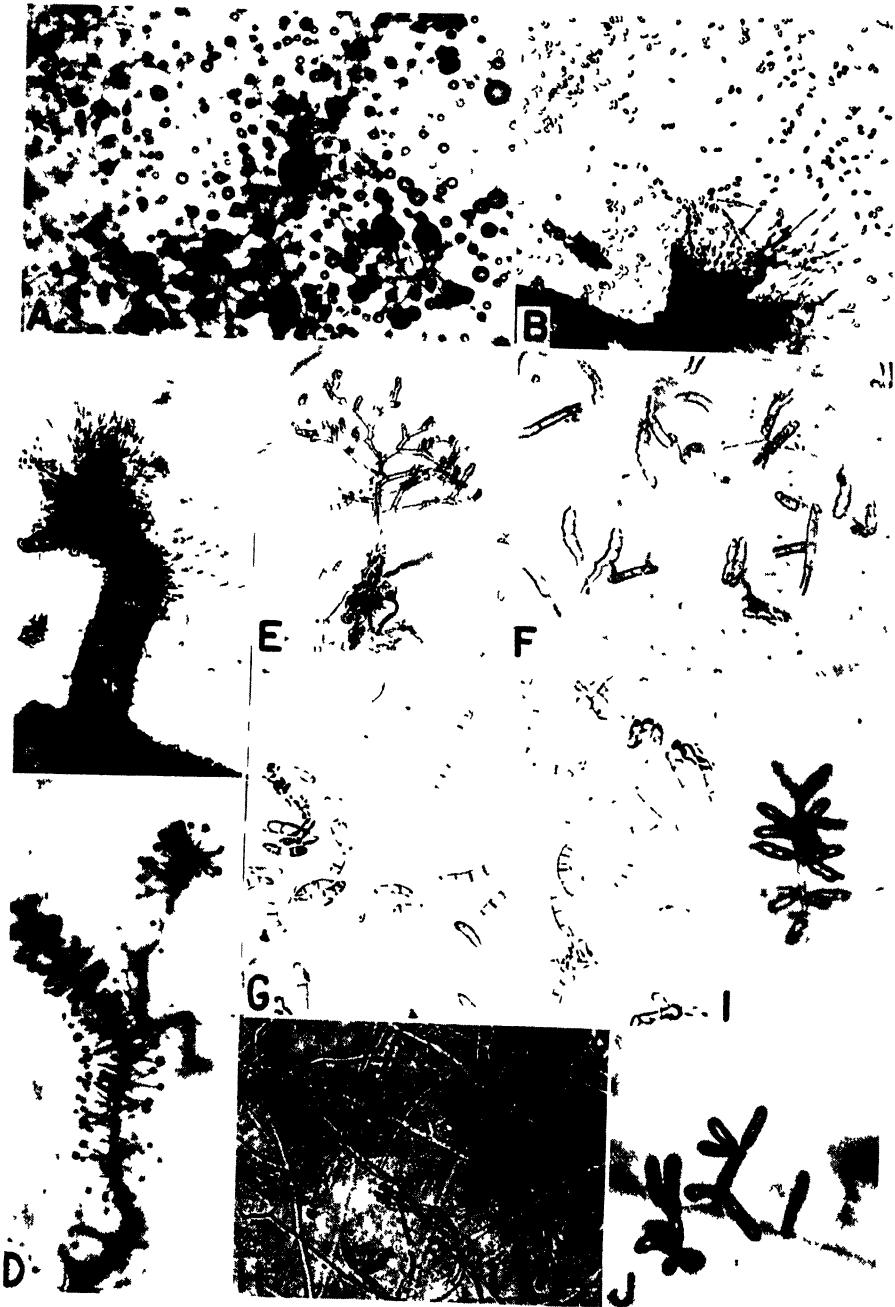
FIG. 2. *A–D, Cephalosporium fici.* A, Cephalo-porium heads in water droplets on string bean agar, 14×; B, conidia directly from fig leaf, 146×; C, leaf hair with fungus growing over it, 146×; D, ropes of hyphae with phialides and spore heads developed on potato-dextrose agar, 62×. *E–J, Ormathodium fici* (E to G, directly from fig leaf). E, conidiophore with typical branching, 140×; F, germinating conidia, 280×; G, conidia, 280×; H, mycelium and conidia from water agar culture, 280×; I–J, conidia attached to conidiophores, developed on aerial mycelium in water agar culture, 280×.

semi-erecta et pila foliorum circumplectentia praecipue se sustenent. Conidiis paucis vel multis in racemis ad apices conidophorum ac a latere in nodis per typum effectis, singulis exsistentibus aut in seriebus brevibus duo vel tria numero continentibus, cylindricis vel clavatis, rectis usque ad formam perspicue curvatam, subbrunneis, 1–5 (–7) septatis, levibus vel per occasionem minute verruculosis 3.4–5.2 × 13.9–29.6 μ, hila minuta in finibus proximis gerentibus, potestatem tubuli germinum e quaque cellula efficiendi habentibus.

Parasiticum in foliis *Fici caricae.*

Spots large, brown, appearing somewhat concentrically zoned on the upper leaf surface and covered with a grayish fuzzy growth of the fungus on the lower surface, dead parts of the lesions frequently falling out. Mycelium within the leaf intercellular, giving rise to the external mycelium through the stomates; external mycelium light brownish, 2–4.5 μ in diameter. Conidiophores arising chiefly from the external mycelium, brownish, branched, multiseptate, with conspicuous nodes on which the conidia are typically borne in groups, semi-erect and supporting themselves mainly by twining around the leaf hairs, measuring 3–4 × 135–240 μ. Conidia typically produced in clusters of few to many at the tips of the conidiophores and laterally on the nodes, arising singly or occasionally in short series of 2–3, leaving minute wart-like scars at the points where they are shed from the nodes, cylindrical or clavate, straight to distinctly curved, light brownish, 1–5-(rarely up to 7–) septate, the majority 3-septate, smooth or occasionally becoming minutely warted, measuring 3.4–5.2 × 13.9–29.6 μ, possessing a minute hilum at the proximal end, producing a germ tube from any cell.

Parasitic on leaves of *Ficus carica;* E. C. Tims, Schriever, Louisiana; September 17, 1947.

Cultural characters

Ormathodium fici was isolated by making spore dilutions with string bean agar. The conidia germinated rather slowly at room temperature. The germinating spores were picked up from the culture plates and transferred to agar slants. Numerous attempts were made to isolate the fungus from small pieces of fig leaf with mycelium attached, but without success.

The fungus grew slowly on agar media. Colonies grown at 28°–32° C. for 38 days on oat meal agar were about 5 cm. in diameter. Growth was somewhat faster at 22°–25° C. The aerial mycelium was almost pure white. But the colonies on oat meal agar were light to dark brown. Sporulation was very sparse on most culture media. Occasionally conidia were observed in short chains of 2 or 3. Many cultures failed to produce conidia during several months of growth on a number of different media. Some sporulation occurred on water agar plates (Fig. 2, H–J). Certain strains of the fungus that produced no conidia in culture proved to be parasitic on fig leaves, where normal sporulation took place.

Taxonomy

The genus *Ormathodium* was erected by Sydow (1928) to accommodate a parasitic *Dendryphium*-like fungus. As was recently pointed out by one of the writers (3), *Dendryphium* was founded on a saprophytic species and practically all species placed in the genus since then have been saprophytes. Both *Dendryphium* and *Ormathodium* are dematiaceous genera characterized by cylindrical, several-septate conidia borne in chains or sometimes singly on branched conidiophores. *Ormathodium* is distinct primarily in its decidedly parasitic nature.

The new species on fig leaves has a large percentage of its conidia borne singly on the conidiophores, but conidia are also occasionally found in short chains of 2–3 on the leaves and more frequently so in the cultures. The fungus might fit just as well into the genus *Dendryphiella*, which has conidia borne singly on nodes of the conidiophores, but this genus was erected to accommodate a saprophytic species. The writers are aware of the fact that genera cannot always be adequately separated on the basis of parasitism and saprophytism. Nevertheless, it is believed that these factors should be employed in the taxonomy of Fungi Imperfecti wherever they can be used to advantage. The new species is therefore included, in the genus *Ormathodium*, at least until a better system of classification for this group of fungi has been devised.

The present fungus in many respects resembles *Ormathodium ambrosiae* Olive (3), recently described as a parasite on leaves of *Ambrosia trifida* in Louisiana. The latter species is characterized by the presence of very similar conidiophores supported by the leaf hairs and by having 1–5-septate conidia borne singly or in short chains of 2–3. Other characters, such as symptoms, cultural characteristics, and conidial size, show that *O. ambrosiae* is distinct from the species on fig leaves. A search through the literature has failed to reveal any parasite of the fig with characters of the present fungus.

Pathogenicity

Inoculations were made on fig leaves in the following manner. The fungus was grown on oat meal agar slants for about 3 weeks, after which the agar and fungus were macerated and made into a paste with the addition of sterile water. Small amounts of this material were placed on the lower surfaces of fig leaves, which were kept moist for 48 hours. Typical spots developed in 10 days to 2 weeks. The leaf shown in figure 1, D was inoculated by this method. The fungus reisolated from such spots was identical with the original isolate.

Another method of inoculation was as follows: Small sections of naturally infected leaf tissue with the fungus growing on them were attached to the lower surface of healthy fig leaves. The fungus soon grew from the diseased leaf tissue onto the healthy leaves, causing typical spots. All of the inoculations were made on fig trees growing out in the open during late summer months when conditions were favorable for infection.

SUMMARY

Two rather conspicuous leaf spots of fig are described and their causal fungi named. *Cephalosporium fici* nov. sp. causes a large concentric ring spot with white cottony tufts of mycelium and conidia on the lower surface of the leaf. *Ormathodium fici* nov. sp. produces a somewhat similar spot with grayish-brown fungus growth and a definite white border on the lower leaf surface. Both fungi when inoculated onto healthy fig leaves produced the diseases in typical form.

The writers are grateful to Dr. P. G. Moorhead, Head of the Department of Classical Languages, for his preparation of the Latin diagnoses.

Specimens of both of the above described species have been placed in the mycological collection of the United States Department of Agriculture, Beltsville, Maryland, and in the mycological herbarium at Louisiana State University, Baton Rouge, Louisiana.

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PHYSIOLOGIC SPECIALIZATION IN GUIGNARDIA BIDWELLII, CAUSE OF BLACK ROT OF VITIS AND PARTHENO-CISSUS SPECIES[1]

E . S . L U T T R E L L [2]

A disease of Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.), known as black-rot leaf spot, has been recognized for many years. Stevens and Hall (6) mentioned it briefly in 1921, and in 1924 Burger (1) noted its occurrence in Florida. A similar disease was reported on Boston ivy (*Parthenocissus tricuspidata* (Sieb. and Zucc.) Planch.) in the District of Columbia by Edson (2) in 1937. The pathogen associated with each of these leaf spots has been considered to be *Guignardia bidwellii* (Ell.) Viala and Ravaz, the fungus that causes black rot of grapes.

During the past three years black rot has been observed on both Virginia creeper and Boston ivy at several locations in Georgia. Attention was centered on this disease by the complaint of a local nurseryman that muscadine grape stock had been refused certification because of the presence of black rot on Virginia creeper vines adjacent to his vineyard, this being considered a possible source of inoculum for the muscadine grapes. In view of the results of a previous study (3) which demonstrated that cross infection could not be obtained with *Guignardia bidwellii* on native bunch grapes and on muscadine grapes, it seemed unlikely that the fungus on Virginia creeper would serve as a source of inoculum for the disease on muscadine grapes. The investigations described here, therefore, were concerned chiefly with this problem. This report contains the results of cross inoculation experiments with the black-rot fungus on bunch grapes, muscadine grapes, Virginia creeper, and Boston ivy, and also includes a few notes on the black-rot disease on the latter two suscepts.

THE DISEASE

The symptoms of black rot on bunch grapes have been described previously by Reddick (4) and on muscadine grapes by Luttrell (3). It may be recalled that on bunch grapes black rot is primarily a disease of the fruit that results in the mummification of the infected berries. It also produces brown spots on the leaves and black cankers on the stems. On muscadine grapes it is primarily a disease of the vegetative parts characterized by severe leaf spotting and cankers of the stems and peduncles. It rarely occurs on the fruit of resistant varieties; and, even on susceptible varieties, it usually causes only superficial scabs and cankers. It should be noted that the question of whether this difference in symptoms may be

attributed to a difference in the suscept or to a difference in the pathogen
has not been answered. Since the European vinifera grape is susceptible
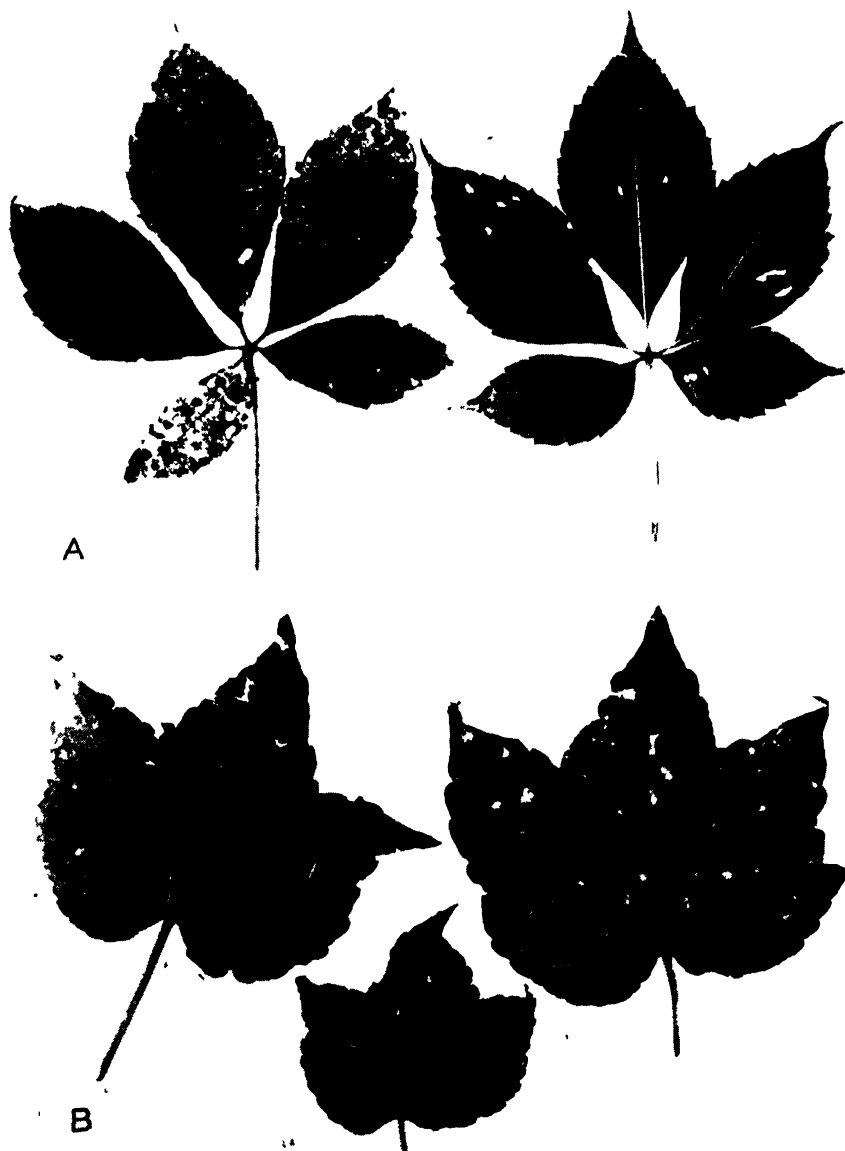
FIG. 1.   A. Black rot spots on leaves of Virginia creeper, at the left from the upper
surface, at the right from the lower surface; × 1/2. B. Black rot spots on leaves of
Boston ivy, the leaf on the left from the lower surface, the two on the right from the
upper surface; × 1/3.

to *Guignardia bidwellii* from both sources, it should be possible to deter-
mine this point by inoculating vinifera grape berries with the pathogen

from both native bunch grapes and muscadine grapes. Unfortunately, mature vinifera grape vines have not been available for this purpose.

The black-rot disease of Virginia creeper and Boston ivy is similiar to that of muscadine grapes in that it is chiefly a disease of the foliage. The leaf spots on Virginia creeper are relatively small, varying in diameter from 3 to 5.5 mm. Generally they do not coalesce, and in natural infections they have not been observed to result in the necrosis of areas of the leaf blade distal to the infected tissue. The spots are dark brown and are surrounded by a broad purplish-black border which merges with the brown center (Fig. 1, A). Pycnidia are produced sparingly on both surfaces of the spots. Brown cankers may sometimes form on the petioles. On Boston ivy the spots are larger, varying in diameter from 5 to 9 mm. They often coalesce and, when the veins are involved, may result in the death of large areas of the leaf beyond the infected tissue (Fig. 1, B). The spots are tan to brown and are surrounded by a narrow purplish-black border. The centers of older spots are often buff-colored. Pycnidia are produced abundantly on both surfaces of the spots. Brown to black cankers are formed on the petioles and on the peduncles of the influorescences. Severe cankers may result in the death of entire leaves, but blight of the inflorescences has not been observed.

Infection of the fruit has been found once on Virginia creeper. Small, brown, superficial, leathery lesions bearing a few pycnidia were present on the infected berries. These symptoms are similar to those on muscadine grape berries. Stem lesions have not been observed on either plant in the field.

Black rot has been found on both wild and cultivated vines of Virginia creeper at both Griffin and Tifton, Georgia. Usually infection is moderate and the leaf spots produced are small. Consequently, the disease is of minor importance and causes little damage even to the appearance of the vines. It has been found on Boston ivy at Griffin and at Atlanta, Georgia, and specimens of diseased leaves have been received from Acworth, Georgia. It is often severe, especially in the spring when nearly all the leaves may be infected and considerable defoliation may result. Since Boston ivy is grown as an ornamental, black rot is important chiefly because of damage to the appearance of the vines.

INOCULATIONS

Inoculations were made on two species of *Parthenocissus* (*P. quinquefolia* and *P. tricuspidata*), the mascadine grape (*Vitis rotundifolia* Michx.), two species of native bunch grape (*V. bourquina* Munson ex Viala and *V. labrusca* Linn.), and the European bunch grape (*V. vinifera* Linn.). The vines to be inoculated were grown in pots in the greenhouse where there was no natural infection. Inoculum consisted of pycnospore suspensions obtained by triturating leaf spots bearing pycnidia collected in the field from *P. tricuspidata, P. quinquefolia, V. rotundifolia,* and *V. labrusca.* In

TABLE 1.—Results of cross inoculations with Guignardia bidwellii on various suscepts.

Suscepts	Source of inoculum															
	Parthenocissus tricuspidata				Parthenocissus quinquefolia				Vitis rotundifolia				Vitis labrusca			
	Shoots		Leaves		Shoots		Leaves		Shoots		Leaves		Shoots		Leaves	
	Inoc.[a]	Inf.[a]	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Parthenocissus tricuspidata	9	88.9	141	18.4	16	75.0	164	32.3	7	0.0	68	0.0	7	0.0	68	0.0
Parthenocissus quinquefolia	7	100.0	104	41.4	8	100.0	88	68.2	7	0.0	95	0.0	8	0.0	98	0.0
Vitis rotundifolia	9	0.0	61	0.0	8	0.0	50	0.0	2	100.0	14	42.9	3	0.0	30	0.0
Vitis bourquina	12	0.0	112	0.0	5	0.0	33	0.0	4	0.0	22	0.0	2	100.0	8	37.5
Vitis labrusca	5	0.0	28	0.0	3	0.0	14	0.0			14	0.0	3	100.0	18	16.7
Vitis vinifera	7	0.0	34	0.0	4	0.0	34	0.0	1	100.0	8	75.0	1	100.0	9	55.6

[a] Inoc. = inoculated; Inf. = infected.

addition, inoculations were made with suspensions of pycnospores produced in culture on 3 per cent malt agar by ascospore isolates obtained from over-wintered leaves of *P. tricuspidata* and *P. quinquefolia*. The inoculum was applied with a pipette to all of the leaves of young, rapidly growing shoots. The inoculated plants were then placed in a moist chamber for 48 hours. Symptoms appeared after an incubation period of two to three weeks. On all suscepts only leaves that were immature at the time of inoculation became infected.

The results of these inoculations are presented in table 1. Inoculum from either Virginia creeper or Boston ivy infected both of these suscepts but no species of grape. Conversely, no infection of Virginia creeper or Boston ivy was obtained with inoculum from either muscadine or bunch grapes. On native grapes, inoculum from muscadine grape produced infection only on muscadine grape; inoculum from bunch grape produced infection only on bunch grape. The introduced European bunch grape, however, was susceptible to infection with inoculum from either muscadine grape or native bunch grape.

<div align="center">TAXONOMY</div>

In a previous report (3) it was shown that *Guignardia bidwellii* occurs on muscadine grapes as a distinct race differing in pathogencity from *G. bidwellii* on bunch grapes. These two races differed morphologically in size of ascospores and pycnospores. In spite of these differences the fungus on muscadine grapes was given taxonomic rank as a form of *G. bidwellii*. From the results of the inoculations reported here (Table 1) it is apparent that *G. bidwellii* exists in three physiologic races: one pathogenic to native bunch grapes, one to muscadine grapes, and one to *Parthenocissus* species. A consideration of morphology (Table 2) supports the classification of these physiologic races as forms of the one species *G. bidwellii* rather than as distinct species. While the race on muscadine grapes is distinct from the other two races in mean width of ascospores, the mean lengths of ascospores for the three races form an intergrading series, the ascospores of the race on *Parthenocissus* species being intermediate and closing the gap between the other two races. Within the race on *Parthenocissus* species there appears to be a difference in mean length of ascospores produced on the two susceptible species, *P. tricuspidata* and *P. quinquefolia*. The differences in size of pycnospores of the three races are even less than those in size of ascospores; and reference to data published previously (3) shows that the variation among sets of measurements of pycnospores within the same race may be as great as the variation among different races. Although there seems to be a definite tendency toward the production of larger ascospores and pycnspores in the race on muscadine grapes, the differences are too subtle to be of practical use in the separation of these races.

In type of colony produced in culture, the race on *Parthenocissus* species again is intermediate between the race on bunch grapes and the race

on muscadine grapes. On 3 per cent malt agar, the race from bunch grapes produced a white, rapidly growing mycelium that became gray or tan with age. The pycnidia were scattered over the mycelium. In the race from muscadine grapes, the mycelium was black and formed a hemispherical, stromatic mass on which the pycnidia were densely grouped. Growth was never extensive. Colonies of the race from *Parthenocissus* species were similiar to those of the muscadine race in that the mycelium was black and the pycnidia were densely grouped on the surface. The growth was much more extensive, however, forming a flat, wide-spread subiculum on the surface of the agar.

Although the race on *Parthenocissus* species was intermediate between the other two races in morphology and in growth in culture, it showed a greater difference in pathology. While the race on muscadine grapes and

TABLE 2.—*A comparison of spores produced by Guignardia bidwellii on different suscepts*

Suscept	Ascospores		Mean[a]	Pycnospores		Mean[b]
	Range microns		microns	Range microns		microns
Vitis labrusca	10.6–16.5 × 5.3–8.0		13.15 × 6.55	7.5–10.9 × 5.4–7.5		8.42 × 6.78
Parthenocissus quinquefolia	11.7–16.0 × 5.3–8.0		13.70 × 6.55	7.5–10.2 × 5.4–8.2		8.53 × 6.74
Parthenocissus tricuspidata ..	10.9–18.4 × 4.8–7.9		14.50 × 6.50	7.1–10.9 × 6.5–8.6		8.46 × 7.30
Vitis rotundifolia .	12.6–17.3 × 6.0–9.0		14.90 × 7.10	7.2–13.9 × 5.4–8.2		9.26 × 6.93

a 200 spores.
b 100 spores.

the race on native bunch grapes have a common host in *Vitis vinifera,* it was impossible to obtain infection of this species with the *Parthenocissus* race.

In view of the intergradations in morphology, it is proposed that the fungi on bunch grapes, muscadine grapes, and *Parthenocissus* species continue to be referred to the single species, *Guignardia bidwellii,* and that within this species three forms, separated upon the basis of pathogenicity, be recognized.

Guignardia bidwellii (Ell.) Viala and Ravaz

Ascocarps separate, black, spherical, 61–199 μ diam., innate, erumpent and ostiolate at the apex, on overwintered berry mummies and dead leaves; centrum pseudoparenchymatous; asci fasciculate, aparaphysate, cylindrical to clavate, 36–56 × 12–17 μ, short-stipitate, 8-spored; ascus wall thick, composed of two layers; ascospores hyaline, non-septate, ovoid or oblong, straight or inequilateral, rounded at the ends, biseriate, 10.6–18.4 × 4.8–9.0 μ.

Pycnidia black, spherical, 59–196 μ diam., innate, erumpent and ostiolate at the apex; produced on the host during the growing season; on leaf blades amphigenous in circular, reddish-brown, necrotic spots; on stems,

tendrils, peduncles, and petioles in elliptical to elongate, brown to black cankers; on fruits either in berry mummies or in brown to black superficial scabs and cankers; pycnospores hyaline, non-septate, ovoid to oblong, rounded at the ends, 7.1–14.6 × 5.3–9.3 μ.

Spermogonia black, spherical, 45–78 μ diam., innate, erumpent and ostiolate at the apex, produced toward the end of the growing season on berry mummies or dead leaves in association with ascogonial stromata; spermatia hyaline, non-septate, bacilliform, about 2.5 × 1 μ.

On *Vitis* and *Parthenocissus* species.

1. **Guignardia bidwellii f. euvitis** f. nov.   Pathogenic to American species of Euvitis and to *V. vinifera*.
2. *Guignardia bidwellii* f. *muscadinii* Luttrell (Phytopath. 36: 913. 1946.) Pathogenic to *Vitis rotundifolia* and *V. vinifera*.
3. **Guignardia bidwellii f. parthenocissi** f. nov.   Pathogenic to *Parthenocissus* species.

The genetic connection of the perfect and imperfect stages of the forms *euvitis* and *muscadinii* has been demonstrated by previous inoculations (3). The present inoculations with pycnospores from ascospore isolates of the form *parthenocissi* establish the same point for this form.

DISCUSSION

Results of these inoculations show that *Guignardia bidwellii* comprises three physiologic forms: f. *euvitis* pathogenic to native bunch grapes, f. *muscadinii* pathogenic to muscadine grapes, and f. *parthenocissi* pathogenic to *Parthenocissus* species.   Infection from cross inoculations with these three forms on the three groups of suscepts could not be obtained.   It seems unlikely, therefore, that black rot on either Boston ivy or Virginia creeper may serve as a source of inoculum for the disease on muscadine grapes and bunch grapes.

The recognition of physiologic forms in *Guignardia bidwellii* is not without precedence in the Ascomycetes.   Physiologic specialization has been reported in many species of the Erysiphales, Hypocreales, Sphaeriales, and Dothideales (7) ; and it seems desirable to give these races differing in pathogenicity the taxonomic rank of form.   In the Mycosphaerellaceae physiologic specialization has been reported previously by Roark (5) who found that *Mycosphaerella rubi* could be separated into two races, one pathogenic to blackberry, the other to raspberry.   To this may be added the present report on *Guignardia bidwellii*.   Since species in *Mycosphaerella* and *Guignardia* have frequently been based on host differences, it is probable that a taxonomic revision of these genera would result in a reduction in number of species and the recognition of physiologic forms in many additional species.

SUMMARY

Cross inoculations have shown that *Guignardia bidwellii* (Ell.) Viala and Ravaz, cause of black rot of bunch grapes, muscadine grapes, Virginia

creeper (*Parthenocissus quinquefolia* (L.) Planch.), and Boston ivy (*P. tricuspidata* (Sieb. and Zucc.) Planch.), comprises three physiologic races differing in pathogenicity. Although slight differences in morphology and in growth in culture accompany these differences in pathogenicity, the three races are classified as forms of *G. bidwellii* as follows: *G. bidwellii* f. *euvitis* f. nov., pathogenic to native bunch grapes and (*Vitis labrusca* Linn., *V. bourquina* Munson ex Viala) and to *V. vinifera* Linn.; *G. bidwellii* f. *muscadinii* Luttrell, pathogenic to muscadine grapes (*V. rotundifolia* Michx.) and to *V. vinifera;* and *G. bidwellii* f. *parthenocissi* f. nov., pathogenic only to *Parthenocissus* spp. Because of physiologic specialization in *G. bidwellii* it seems unlikely that the fungus on Virginia creeper and Boston ivy may serve as a source of inoculum for the black rot disease on either muscadine or bunch grapes.

GEORGIA EXPERIMENT STATION,
EXPERIMENT, GEORGIA

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STUDIES OF TWO VIRUSES CAUSING MOSAIC DISEASES OF SOYBEAN[1]

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INTRODUCTION

The great expansion of soybean (*Soja max* Piper) culture in recent years has led to a large-scale breeding program, and to an increased study of the diseases of this crop. In 1916 Clinton (1) described the symptoms of soybean mosaic; in 1921 Gardner and Kendrick (2) established the virus nature of the disease, and in 1924 they (7) showed that the virus was transmitted from generation to generation through the seed of infected plants. Since that time almost no work has been done on the disease in this country. Heinze and Köhler (4) determined the properties and demonstrated aphid transmission of a virus causing soybean mosaic in Germany. Observations by a number of pathologists indicate that soybean mosiac is present in all major soybean producing areas, and that it causes losses in both quality and quantity of seed.

Certain of the more tolerant varieties of soybean which rarely show symptoms under field conditions have come to be regarded as "resistant" to mosaic. The feeling has arisen that these tolerant varieties maintain their "resistance" as long as they are grown in their areas of adaptation; however, when grown in different regions, they appear to be susceptible. If it were true that a mere change of location would alter the resistance of a group of varieties, plant breeders would be confronted with serious mosaic problems. It is a common practice for crosses to be made between varieties adapted to widely different environments.

Wide differences in mosaic symptoms seen in the field suggested that more than one virus might well be responsible for the mosaic disease of soybean. Some pathologists (8, 10) have shown that certain leguminous viruses are capable, under experimental conditions, of causing systemic infection of soybean. This paper reports the results of an investigation made to clarify a number of these aspects of our knowledge of soybean mosaic.

MATERIALS AND METHODS

Thirty-two virus isolates were obtained from naturally infected, field-grown soybeans in the summer of 1946. These were differentiated by inoculation to soybean, tobacco (*Nicotiana tabacum* L., Havana type No.

38), and garden bean (*Phaseolus vulgaris* L., variety Burpee's Stringless). Fifteen of the isolates were proved by the reaction on tobacco to be the tobacco ring-spot virus commonly found on soybean and recognized as the cause of bud blight. These were discarded.

Fifteen of the isolates were infectious only to soybean. These isolates, which tests indicated were separate isolations of the same virus, produced symptoms agreeing with those described by Clinton (1), and Gardner and Kendrick (2), and the seed from infected plants produced diseased seedlings. This virus will be referred to subsequently as *Soja virus 1* and the disease caused by this virus on soybean as soybean mosaic.

Two of the virus isolates were infectious to garden beans in addition to soybeans. These reacted identically and were considered separate isolations of the same virus. Tests, reported later in this paper, show this virus to be a strain of *Phaseolus virus 2*, the virus causing yellow mosaic of garden bean. This virus will be referred to as *Phaseolus virus 2* and the disease caused on soybean as yellow mosaic.

The soybean variety Bansei was used throughout the investigation as the "standard" variety. The soybean seed used was provided by the U. S. Regional Soybean Laboratory, Urbana, Illinois. Except for the variety Cherokee all seeds were free from mosaic. Other seeds were provided by the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, or were purchased from reputable seedsmen.

Except for some field studies of seed transmission, all experimental work was done in the greenhouse at temperatures which usually varied between 18° C. and 24° C. Supplemental light from 500-watt incandescent bulbs was supplied during the winter period of low intensity. In the design of the experiments and interpretation of results the possibility of accidental insect transmission of the viruses was constantly considered and adequate precautions were taken.

Soybeans were inoculated on both primary leaves when the first trifoliate leaf was less than one-fourth inch long. Carborundum was used in all inoculations. Virus properties were determined according to usual methods, each test being repeated from three to five times.

EXPERIMENTAL RESULTS

Symptomology. On Bansei soybeans the first visible symptom of disease, following mechanical inoculation with *Soja virus 1*, occurs as a yellowish vein-clearing which develops in the minor veins of the developing trifoliate leaves. This symptom is transitory and appears from 6 to 14 days after inoculation. Rugose symptoms usually appear on the third trifoliate leaf formed after inoculation, increase in severity on successive leaves and eventually becomes dark green, puffy areas among the major veins of the leaf (Fig. 1, A). These vesicles, formed by the upward proliferation of the blade, may be scattered or aligned on either side of
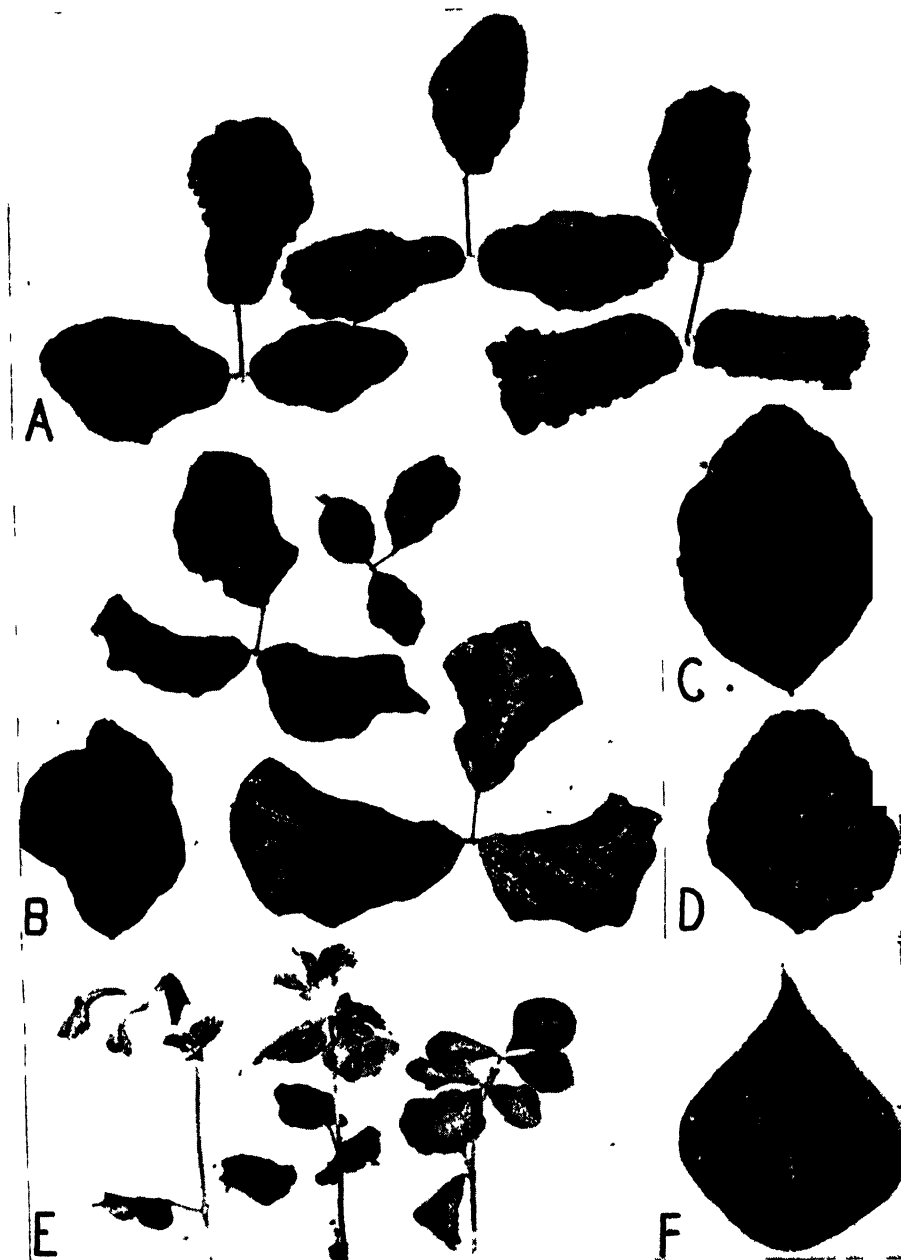
FIG. 1. Symptoms of soybean mosaic on Bansei (A) and Flambeau (B) soybeans. Yellow mosaic symptoms on Bansei (C) and Flambeau (D) soybeans. E. Symptoms produced by seed born *Soja virus 1* on Lincoln seedlings. F. Mottling of Burpee's Stringless garden bean leaflet by *Phaseolus virus 2* isolated from soybean.

the major veins. Leaflets may be yellowed among the vesicles and around the margin. Vein-clearing of the matured leaves is common. Leaf margins frequently are curved downward at the sides and upwards at the tip. Leaves become coarse, leathery to the touch, and somewhat brittle at maturity. Diseased plants are stunted and appear to set fewer pods than normal plants. Pods borne on diseased plants are mostly normal; however, some may be curved laterally or twisted and have less conspicuous pubescence. Many pods produced on diseased plants do not bear seed. With the exception of the variety Ogden, the reaction of the tested oil-type varieties is uniform and much less severe than the reaction of Bansei. Leaves of the oil-type varieties are somewhat wrinkled but the conspicuous dark green vesicles characteristic of the disease on Bansei are lacking. Margins of the leaflets tend to be wavy, and bend downward or roll inward in an irregular manner (Fig. 1, B). Occasionally the leaves have a slight yellowish mottle.

The reaction of the Ogden variety to *Soja virus 1* is different from the reactions of the other varieties tested. From 10 to 20 per cent of the plants develop a progressive necrosis which ultimately reaches the terminal bud. The leaves produced on occasional branches are severely dwarfed, mottled, distorted, and dotted with necrotic spots. Since this type of reaction approximates the reaction of soybeans to the tobacco ring-spot virus, inoculations were made from such plants to tobacco to rule out the possibility of accidental tobacco ring-spot virus infection. Tobacco plants were unaffected by the inoculation, and transfers from the inoculated tobacco to Bansei were negative indicating that the virus was not present in masked form in tobacco. Bansei seedlings, inoculated with juice from such Ogden plants, had symptoms typical for *Soja virus 1*.

No marked distinction between vegetable and oil-type soybeans, or between varieties, was observed as a result of infection with *Phaseolus virus 2*. Initial symptoms occur essentially as described for *Soja virus 1;* however, they are less transitory. Successive leaves have a conspicuous yellow mottle as the characteristic symptom (Fig. 1, C and D). The yellow areas may be scattered or occur as indefinite bands along the sides of the major veins. Under certain conditions intense yellow islets appear in the blade. As the leaves mature, rusty necrotic spots develop in the yellowed areas. Diseased plants as a rule are not markedly stunted, and, in the greenhouse, pod set was not appreciably diminished. No symptoms were observed on the pods.

Effect of air temperature on symptom expression. Observations that soybean mosiac is more severe in certain parts of the growing season suggested that the symptomology of the disease is correlated with temperature. Johnson (6) gave some indication of the relationship when he stated ". . . soybean mosaic was completely inhibited at temperatures of 26–28° C." In order to obtain a more complete picture of the relationship a further investigation was made. Three constant temperature cabinets

set at 18.5° C., 24° C., and 29.5° C. were used for this purpose. It was thought that varieties adapted to a growing region with a certain mean temperature might react similarly to each other and differently from varieties adapted to other regions with different mean temperatures. The soybean varieties used to test this possibility included Mandarin and Habaro, northern varieties; Lincoln and strain A4–107–12[3], central types; Louisiana Green and Cherokee, southern varieties. In addition, the vegetable variety Bansei, a mid-season variety in Illinois, was included. Test plants were inoculated and left at usual greenhouse conditions until the initial symptoms of mosaic developed, after which they were transferred to appropriate cabinets. Noninoculated plants of the same age and subjected to the same environment were included in all tests. At least nine plants of each variety were used in each test, and all tests were repeated.

The first two or three leaves formed on seedlings inoculated with *Soja virus 1* were much alike at all temperatures, and were similar to symptoms developing on duplicate plants left in the greenhouse. Subsequent leaves, however, showed striking differences at the various temperatures. This was most spectacular with the variety Bansei, but was evident with all other varieties except Cherokee in which there was only a mild response. Infected Bansei plants grown at 18.5° C. for four weeks were markedly stunted in leaf size and plant height. Leaflets were extremely rugose and were curled upward around the margin. Pubescence on both stem and leaves was extraordinarily apparent. Symptoms on infected Bansei plants at 24° C. were much less severe. Leaflets were considerably distorted, and spotted with raised, dark green swollen areas. Mottling was also apparent at this temperature. Infected Bansei plants grown at 29.5° C. had very mild or no recognizable symptoms (Fig. 2, A). Some leaflets were obscurely mottled, occasionally margins were wavy, but no rugosity or stunting was apparent. The oil-type varieties infected with *Soja virus 1* reacted rather uniformly but did exhibit some varietal differences not correlated with areas of adaptation. Leaflets of Lincoln grown at 18.5° C. were rolled under and inward, misshapen, and considerably wrinkled; however, they lacked the extreme rugosity characteristic of Bansei at this temperature. At 24° C. leaflets were mottled, had wavy margins, but no crinkling of the blade. At 29.5° C. infected plants were essentially symptomless (Fig. 2, B). Habaro, Mandarin, and strain A4–107–12 reacted as did Lincoln. Louisiana Green was more severely dwarfed and distorted than Lincoln at 18.5° C., and the leaflets were mottled at 29.5° C. Cherokee was mildly affected at 18.5° C. and without noticeable symptoms at other temperatures. The varieties adapted to a given region did not react as a group. There was as much variation within the varieties adapted to a certain region as there was between those of different regions. Inoculations to healthy Bansei seedlings from the infected, symptomless plants of all varieties grown at 29.5° C. resulted without exception in the recovery of the virus.

[3] One of the sister strains composited to make the variety Hawkeye.
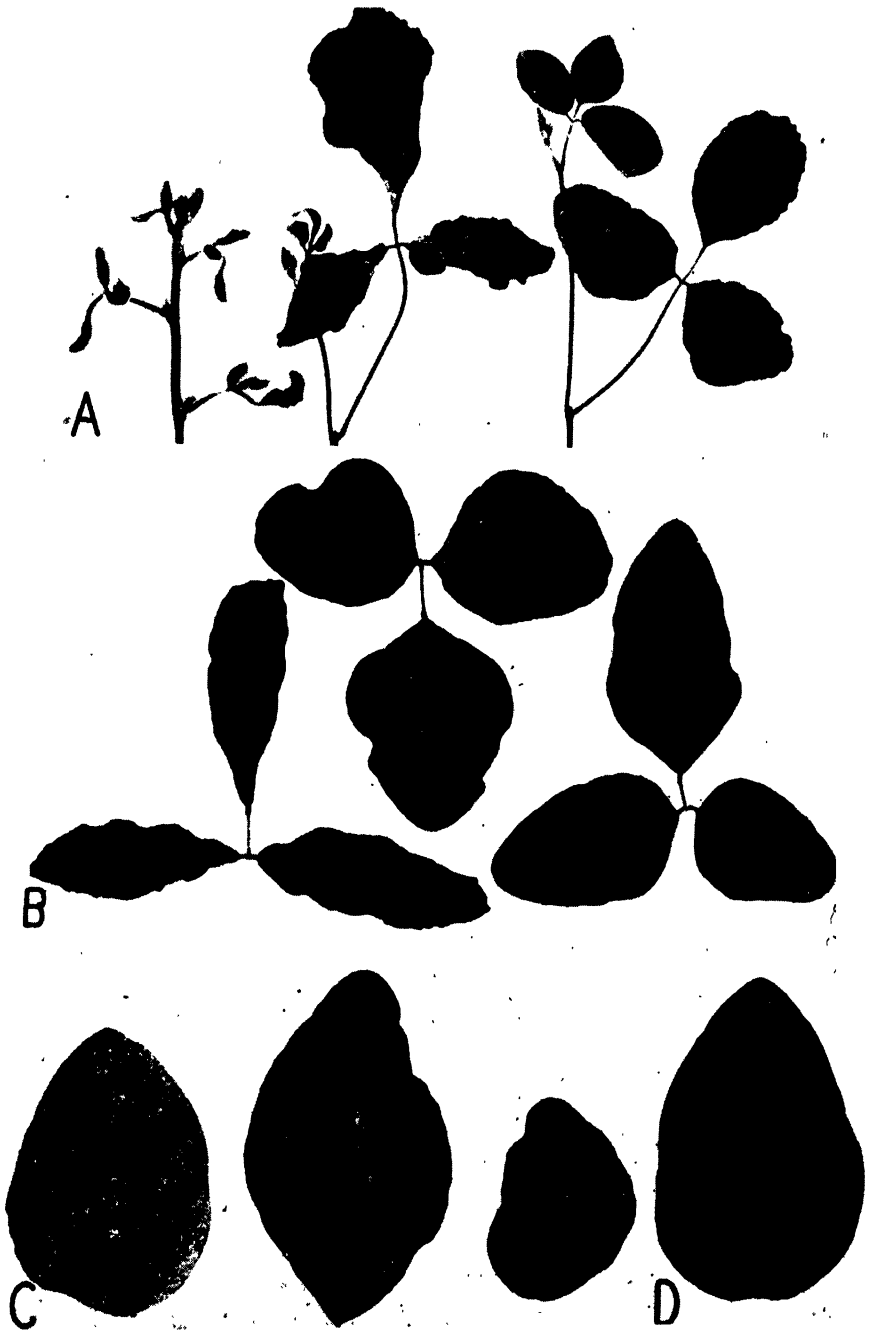
FIG. 2, A–C. Mosaic symptoms on soybeans after 31 days in constant temperature cabinets at (from left to right) 18.5° C., 24° C., and 29.5° C. A. Stem tips of variety Bansei infected with *Soja virus 1*. B. Leaves of Lincoln variety infected with *Soja virus 1*. C. Leaflets of Bansei variety infected with *Phascolus virus 2*. D. Healthy Bansei leaflet.

The symptomology of soybeans infected with *Phaseolus virus 2* was found not to vary conspicuously with either temperature or variety. Yellow mottling was obvious at all temperatures used; however, a slight difference was noted in the type of mottling. At 18.5° C. mottling on Bansei was of a rather diffuse nature, while at 29.5° C. the yellowed areas were fewer and larger (Fig. 2, C). A slight puckering tendency around the yellowed areas was noticeable at the latter temperature. Oil-type varieties reacted similarly to Bansei; the varieties Habaro and Mandarin were noticeable in that rusty, necrotic spots developed abundantly on the matured leaves of plants grown at 18.5° C.

Temperature was found to have a considerable effect on the incubation period of both viruses. *Soja virus 1* symptoms on Bansei appeared in four days at 29.5° C., in six days at 24° C., and in fourteen days at 18.5° C. *Phaseolus virus 2* symptoms appeared in five days at 29.5° C., seven days at 24° C., and twelve days at 18.5° C.

Host range studies. Table 1 presents data showing the species tested as hosts and results of inoculations with *Soja virus 1* and *Phaseolus virus 2*. Attempts were made, in most cases, to recover the viruses from both the inoculated leaves and from leaves formed subsequent to inoculation. *Soja virus 1* did not produce symptoms on any of the species tested; however, the virus was recovered from the symptomless, inoculated leaves of the Burpee's Stringless, Stringless Green Pod, and Stringless Green Refugee varieties of garden beans 23–27 days after inoculation. The virus was not recovered from the trifoliate leaves of these varieties; and it was not recovered from either the inoculated or trifoliate leaves of the Corbett Refugee and U. S. #5 Refugee varieties. The virus was not recovered from any other species tested.

Inoculation of the various species tested as hosts of *Phaseolus virus 2* resulted in systemic mottling in the following: white and yellow sweet clover, crimson clover, broad bean, Alaska and telephone varieties of peas, and all the varieties of garden beans tested. The virus was recovered only from plants showing symptoms. The first symptom observed in garden beans was a drooping of the leaflets at the pulvini; however, this was not obvious in all varieties. Later small halo-like spots appeared in the blades which enlarged and coalesced to form a conspicuous yellow mottling (Fig. 1, F). Mottling became more distinct as the leaves matured, and at maturity leaflets were cupped downward and appeared rather glossy. Affected plants were not markedly stunted and no symptoms were produced on the pods.

Physical properties of the viruses. Thermal inactivation point determinations were carried out by placing 1 ml. of inoculum (sap diluted with an equal volume of distilled water) in thin-walled, small glass tubes and heating for 10 minutes in a water bath. In longevity in vitro tests the inoculum (undiluted sap) was placed in stoppered test tubes and stored

at 18.5° C. in diffuse light.   All property tests were repeated at least three
times except the longevity in vitro test with *Phaseolus virus 2* which was
performed twice.

TABLE 1.—*Results of inoculations with Soja virus 1 and Phaseolus virus 2 with various species of plants*

Species tested	Results of inoculation with			
	Soja virus 1 No. of plants		*Phaseolus virus 2* No. of plants	
	Inoculated	Infected	Inoculated	Infected
Crotolaria spectabilis Roth. ...	16	0a	16	0
Lespedeza striata (Thumb.) H. & A. .	13	0b	21	0
Lupinus angustifolius L., blue lupine	18	0a	12	0
L. luteus L., yellow lupine	10	0a	16	0
Medicago sativa L., alfalfa	14	0b	29	0
Melilotus alba Desv., white sweet clover	14	0b	20	12
M. officinalis (L.) Lam., yellow sweet clover	14	0b	10	4
Phaseolus aureus Roxb., mung bean	15	0	15	0
P. coccineus L. scarlet runner bean	7	0	15	0
P. lunatus L., civet bean, Henderson Bush variety	4	0a	14	0
P. mungo L., urd bean	15	0	15	0
P. vulgaris L., garden bean				
Asgrow Black Valentine variety			16	14
Burpee's Stringless variety ...	98	0c	20	18
Corbett Refugee variety	5	0	20	8
Full Measure variety ...			19	12
Improved Golden Wax variety			11	10
Red Kidney variety			12	8
Stringless Green Pod variety	9	0c		
Stringless Green Refugee variety	13	0c	20	17
Stringless Red Valentine variety			14	13
U. S. No. 5 Refugee variety	4	0	20	12
Pisum sativum L., pea,				
Alaska variety			16	13
Telephone variety			14	11
Trifolium incarnatum L., crimson clover	14	0a	14	13
Vicia faba L., broad bean			6	6
Vigna sesquipedalis (L.) Fruwirth, asparagus bean	15	0	15	0
V. sinensis (L.) Endl., cowpea				
Victor variety	15	0	15	0
Whippoorwill variety ...	15	0	15	0
Beta vulgaris L., beet, Detroit Dark Red variety	17	0	27	0
Lycopersicon esculentum Mill., tomato				
Early Baltimore variety	11	0	4	0
Nicotiana tabacum L., tobacco				
Havanna type No. 38 ...	39	0	12	0

a No attempt made to recover *Soja virus 1*.

b No attempt made to recover *Soja virus 1* from inoculated leaves; however, recovery
attempts from tops were negative.

c *Soja virus 1* recovered from symptomless, inoculated leaves 23–27 days after inoculation.

Soja virus 1 had a thermal inactivation point lying between 64° C. and
66° C.   The virus was still infectious after four days aging in vitro, but
was non-infectious after five days.   The results of five attempts to establish
the tolerance to dilution varied so widely that no definite value was attained.

The thermal inactivation point of *Phaseolus virus 2* was determined to be between 54° C. and 56° C. The virus had a longevity in vitro of three to four days. The dilution end-point varied so widely in five tests that no definite value was obtained.

Seed transmission studies. It has been demonstrated that soybean mosaic is seed transmitted (4, 7, 8). The discovery that *Phaseolus virus 2* causes a field disease of soybeans made the question of whether it is seed transmitted of practical importance. In order to answer this question, and to confirm seed transmission of *Soja virus 1,* numerous naturally infected plants were tagged in the field in the summer of 1946 and the seed from them collected individually. In addition some seed was collected from inoculated plants grown in the greenhouse. This seed was grown in the greenhouse the following winter and in the field in the summer of 1947. Seedlings grown in the greenhouse were observed until flower buds were formed, final readings in the field were made when the plants were approximately 15 inches tall.

Seed from plants[4] tagged in the field as having soybean mosaic produced a relatively high percentage of diseased seedlings, varying from 2 to 75 per cent according to soybean type. Of 1,993 seedlings grown from such seed, 646, or 32 per cent, were diseased. Seed collected from the variety Lincoln, inoculated and grown in the greenhouse, produced 364 seedlings, 15 per cent of which were diseased. Symptoms produced on seedlings by seed-borne *Soja virus 1* were usually observed on the primary leaves. Such leaves had wavy margins and the blade was somewhat crinkled or slightly mottled. The first trifoliate leaf usually showed symptoms characteristic of soybean mosaic on that variety (Fig. 1, E). Seed from several plants tagged in the field as having soybean mosaic failed to produce diseased seedlings.

A total of 362 seedlings were grown from the seed of soybeans inoculated with *Phaseolus virus 2.* None of these were diseased. Seed from plants, mostly strain A4–107–12, tagged in the field produced 6,323 seedlings; none of these had yellow mosaic. Some seed was collected from garden beans inoculated with *Phaseolus virus 2.* The 107 seedlings grown from this seed were all healthy.

Insect transmission. The pea aphid, *Macrosiphum pisi* Kalt.,[5] the peach aphid, *Myzus persicae* Sulzer, and *Thrips tabaci* Lindeman were used in attempts to transmit the viruses by insects. These were cultured on healthy plants of alfalfa, tobacco, and soybean respectively. Aphids were fed on diseased plant parts placed in large diameter glass tubes covered at the ends with several thicknesses of cheesecloth. After feeding 24 to 30 hours on the diseased tissue, the aphids were transferred with usual precautions to healthy seedlings which were enclosed immediately in

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[4] Mostly soybean introductions of United States Department of Agriculture.
[5] Insects were identified by Dr. H. H. Ross, Systematic Entomologist, Illinois State Natural History Survey, Urbana, Illinois.

bell-jars. Under these conditions aphids were dead after five to six days. Similar technique was followed with thrips except that they were collected from diseased soybeans and transferred directly to healthy seedlings in bell-jars.

Both the pea aphid and the peach aphid transmitted *Soja virus 1* to healthy seedlings. In the two tests resulting negatively with the former species only four aphids were transferred to each healthy seedling; ten or more aphids were transferred to each seedling in the six tests resulting positively. Both tests with the peach aphid resulted in virus transmission. Aphids became viruliferous feeding on infected stems, old or young leaves, and stem tips. Four attempts to obtain transmission with thrips were negative. From 50 to 100 thrips were transferred to each healthy seedling. All check plants remained healthy. The incubation time, initial symptoms, and course of development of the disease following infections by insect-transmitted virus was the same as that following mechanical inoculation.

Successful transmission of *Phaseolus virus 2* was obtained only once in nine separate tests with *Macrosiphum pisi*. In this case aphids were fed on diseased garden bean leaves and transferred to garden bean seedlings. Attempts to transmit this virus from soybean and garden bean to soybean were negative. Transmission of this virus with other insects was not attempted.

DISCUSSION

The experimental evidence confirms the observation that temperature plays a large role in the symptomology of soybean mosaic. This also confirms and extends the work of Johnson (6) who reported only that high temperature suppressed symptoms. Tests to determine varietal resistance or immunity should be made at temperatures suitable for symptom expression. It is also clear that the reaction of a variety to *Soja virus 1* is not correlated with the area to which it is adapted; rather the variety is the unit in which the reaction is determined. If there is a danger of creating a soybean variety especially susceptible to soybean mosaic by making crosses between varieties adapted to wide environments the dangerous entity is within the individual varieties used in crossing.

The values obtained for the thermal inactivation point and longevity in vitro of *Soja virus 1* are similar to those obtained by Heinze and Köhler (4). Pierce (8) reported a thermal inactivation point of 56°–58° C. for a seed-transmitted virus causing soybean mosaic; this differs so much from the writer's results that it cannot be presumed the same virus is involved. *Soja virus 1* induced systemic symptoms only on soybeans; this confirms the work of Gardner and Kendrick (2), Pierce (8), and Heinze and Köhler (4). The latter authors reported that the virus produced symptoms and could be recovered from the inoculated leaves of certain varieties of *Phaseolus vulgaris;* the writer was able to recover the virus from the inoculated primary leaves of certain varieties of this species but no symptoms were observed on the inoculated leaves.

The suggestion of some pathologists (5) that soybean "mosaic" is caused by more than one virus is substantiated by this investigation. A second virus was collected from soybean which differed from *Soja virus 1* in symptomology, physical properties, host range, and seed transmission; it closely resembled *Phaseolus virus 2* in physical properties, host range, symptomology, and lack of seed transmission in garden bean. Pierce (8), who found soybean susceptible to *Phaseolus virus 2,* reported collecting the virus from soybeans in the field; however, no description of the disease on soybean was recorded. The virus collected from soybean by the writer differs from *Phaseolus virus 2,* as described by Pierce (8) and Zaumeyer and Wade (10), by not being infectious to mung bean and lespedeza, and by having a thermal inactivation point of 54°–56° C. as compared with 56°–58° C. for *Phaseolus virus 2.* Because of these differences it appears best to regard the virus isolated from soybeans as a strain of *Phaseolus virus 2.*

It has been demonstrated that soybean mosaic spreads in the field (3, 4, 7). Kendrick and Gardner (2) were unable to obtain transmission with the tarnished plant bug and leafhoppers. Heinze and Köhler (4) found that, in Germany, eight species of aphids, among them the peach aphid, were capable of transmitting *Soja virus 1.* Transmission of the virus was accomplished in this investigation by the peach aphid and the pea aphid. While the pea aphid has been reported a vector of other legume viruses, this is the first report that it also serves, under experimental conditions, as a vector of *Soja virus 1.* Soybeans were not a favored host of aphids under the conditions prevalent in this study. In the greenhouse aphids rarely increased in numbers or persisted for an appreciable length of time on soybeans. It is believed that aphids may be responsible in part for spread of soybean mosaic in the field. Zaumeyer and Kearns (9) found a parallel situation in their studies on the transmission of common mosaic of bean (*Phaseolus virus 1*). They obtained transmission with a number of aphid species, but found that beans were not a favored host of these insects. They believed that aphids in searching for a more favorable host might feed in the interim on beans, thus spreading the disease. It is likely that under field conditions other insects are capable of transmitting the viruses causing the mosaic diseases of soybean.

SUMMARY

A study was made of two viruses causing mosaic diseases of soybean. The symptomology of soybean mosaic, caused by *Soja virus 1,* and of yellow mosaic, caused by a strain of *Phaseolus virus 2,* was described.

Air temperature had a considerable effect on the symptomology of soybean mosaic, symptoms being severe at 18.5° C., and largely masked at 29.5° C. Soybean varieties were found not to react as groups according to areas of adaptation, but rather as individual varieties. *Soja virus 1* produced systemic infection only on soybeans; however, it was recovered from

the symptomless, inoculated, primary leaves of certain varieties of garden beans. The thermal inactivation point of *Soja virus 1* was from 64° to 66° C.; longevity in vitro 4–5 days. The virus was transmitted through the seed of both inoculated and naturally infected plants. The pea aphid and the peach aphid transmitted the virus from infected to healthy plants; transmission tests with *Thrips tabaci* were negative.

Air temperature did not markedly affect symptom expression of yellow mosaic. All soybean varieties tested reacted similarly to *Phaseolus virus 2*. Besides soybeans, this virus induced systemic mottling on *Phaseolus vulgaris, Vicia faba, Trifolium incarnatum, Pisum sativum, Melilotus alba,* and *M. officinalis*. The thermal inactivation point of this virus was from 54° to 56° C.; longevity in vitro 3–4 days. The virus was not seed transmitted in 6,685 seedlings grown from the seed of inoculated and naturally infected soybeans, or in 107 seedlings from seed of inoculated garden beans.

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COLLAR INJURY OF APPLE TREES ASSOCIATED WITH WATERLOGGED SOIL

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When collar injury appears on apple trees the disturbance is usually attributed either to winter injury or to a pathogen such as *Phytophthora*. Winter injury, however, is often complicated by the condition of poor drainage or a waterlogged soil, thus making it difficult to separate cold injury from that due to poor aeration. The collar injury here described occurred under conditions that apparently precluded winter injury as a contributing factor, and for that reason deserves attention. The observations on collar injury to apple trees herein reported were made during the summer of 1945 on a block of trees at the Plant Industry Station, Beltsville, Md., containing 760 5-year-old trees.

The trees, originally a part of a rootstock experiment, were being used in inoculation tests with *Phytophthora cactorum* (L. and C.) Schroet. As a preliminary treatment prior to inoculation, the area where the trees were growing was given several applications of water (totaling approximately 10 inches) during the month of May. The apparent harmful effects of this irrigation plus the unusually heavy rainfall of 25.1 inches during May, June, and July are reported in this paper.

Inoculation with *Phytophthora cactorum* was attempted by removing the soil from the collar and inserting the inoculum into the root tissue. This was done on 29 trees on May 28, on 161 trees on June 7, and on 40 trees on July 6. The remainder of the trees in the block were left uninoculated as checks. When the soil was removed in preparation for the July 6 inoculation 2 of the 40 trees showed initial stages of collar injury below the soil line. These two trees had not been disturbed by any previous treatment except irrigation. They were not inoculated, but were kept for further observation of the development of the collar injury that was already starting. The progress of the development of the lesions on these 2 trees was studied during the summer.

The lesions on the 2 trees mentioned above were 30 and 40 mm. in diameter, respectively. The spots were brown and water-soaked and their condition indicated that they were of very recent origin.

On August 22 these two trees were dug up and examined and photographed (Fig. 1). By this time the dead area had extended at least half way around one tree and had girdled the other. It had extended downward as far as the branch roots, a distance of 15 cm., but had extended upward very little farther than when the first observation was made in July. The

dead area was water-soaked and in places the bark was gelatinous and slimy
Demarcation between the living and the dead tissues was usually very sharp
Thus the killing started slightly below the soil line and advanced laterally
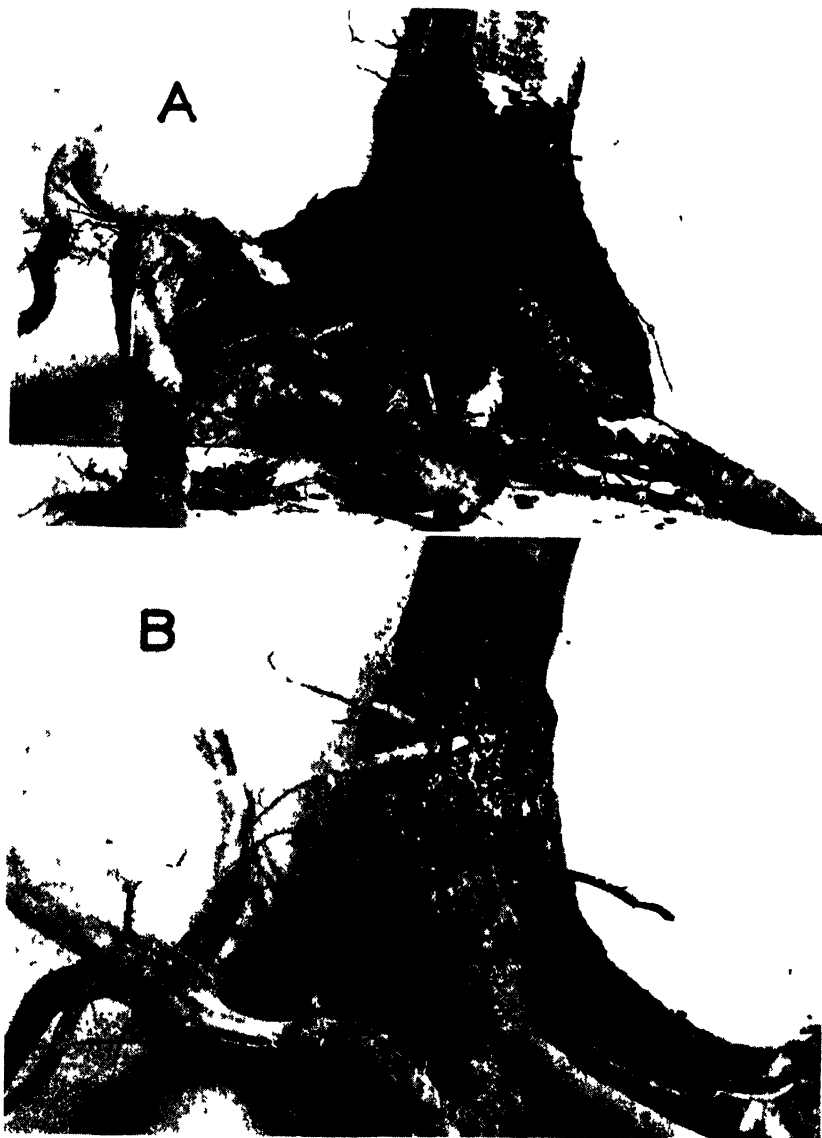
FIG. 1, A.   The extensive dead area was a lesion only 40 mm. in diameter on July 6.
When photographed on August 23 the tree was completely girdled.   B. A companion tree
with one side still alive.   The enlarged lenticels are characteristic of trees growing in a
waterlogged soil.   The bark in the upper part of the picture is discolored but not dead.

and downward rather than upward.   At this time the tops of the trees having
this collar injury showed no abnormal symptoms, which is another indication
that the below-ground dying of the bark was very recent and very rapid.

In general, the killing was uniform throughout the affected bark area. However, one could sometimes see islands of live tissue surrounded by dead tissue. Such islands of living tissue, according to the author's experience, do not occur in lesions caused by an apple root pathogen.

When the first evidence of this disturbance appeared in July and while the lesions were relatively small, thorough culture studies were made, but with negative results. Such studies were made again in August, when the two trees were dug up and photographed. At this time the temperature and media particularly favorable for the growth of *Phytophthora* were provided (2), but no evidence of a pathogenic organism was obtained. Microscopic examination at the margin of the dead and healthy tissue did not show any mycelium. The distinctive character of the lesion as well as the failure to isolate a pathogen and the failure to observe mycelium in newly killed tissue indicates that the collar lesions in question were not due to a pathogen.

In a short time after the injury was noted on the two trees similar lesions were appearing not only on trees used in the inoculation studies but also in

TABLE 1.—*Progressive collar injury to apple trees*

Number of trees examined	Percentage of trees having collar injury			
	May 28	June 7	July 6	November 1
39	0		.	67
161		0		57
38			5	40

the alternate rows that were used as checks for the *Phytophthora* inoculation. The lesions were very irregular in shape and the killing was extremely rapid.

In November a final examination was made of the 238 trees that had been inoculated with *Phytophthora cactorum*. The results are presented in table 1. It will be noted that there was no evidence of injury in May or June, but that incipient injury was showing in July. This table shows that over 50 per cent of the inoculated trees had developed a collar injury typical of that described above for the two trees first observed to have this injury on July 6 and which consequently were not used in the inoculation test. These lesions occurred promiscuously around the collar.

Small lesions developed on a few of the trees (10 per cent) at points where inoculations were made. These lesions were definite in outline but were only 5 to 10 mm. in diameter by November. Reisolation of the pathogen from these small areas was not attempted.

The data in table 1 relate only to trees on which no lesions were found early in the summer, at the time they were inoculated. Examinations were also made in the fall on uninoculated trees that had not been examined earlier in the season. They showed the presence of collar injury at this time, and enough were examined to indicate that the disturbance occurred over the whole block of trees. Observations made on trees that were dead the

following year indicated that collar injury was distributed over the whole block and included both the trees inoculated with *Phytophthora* and the checks for this inoculation test.

The literature contains very little information about collar water injury to pomaceous fruit trees. The few reports that have been made on water injury to fruit trees have been concerned with stone fruits [Fikry (3), Howard (4)], with apple orchards flooded during the winter months [Dorsey and Ruth (1)], and with flooded pear trees [Kienholz (5)]. Pear trees are generally considered more tolerant of a waterlogged soil than apple trees.

SUMMARY

Five-year-old apple trees growing in a heavy soil and having had wet conditions during the summer developed a collar injury during the summer months without the complicating effect of cold injury.

Very small lesions, measuring as much as 10 mm. in diameter, developed on some of the trees inoculated with *Phytophthora cactorum*. Such lesions rarely formed and did not develop further.

The extensive collar injury here described was apparently the result of waterlogged soil.

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A STUDY OF THE FUNGICIDAL ACTION OF 8-QUINOLINOL AND SOME OF ITS DERIVATIVES[1]

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INTRODUCTION

The use of 8-quinolinol and some of its derivatives as fungicides has been mentioned at various times in plant science literature (2, 4, 5, 7, 9, 12, 17, 18, 20, 21, 23, and 24). The 8-quinolinols have been recognized for twenty years or more as having bactericidal properties, but their possibilities as commercial fungicides and plant protectants apparently were not recognized until recently. The cost of the material has been and still is a matter of extreme importance in developing these possibilities. However, Powell (20) has shown that the copper salt of 8-quinolinol has real promise of becoming an important material in the control of scab and blotch of apples. Further field tests at the Illinois Experiment Station have confirmed these first results and it was found that the material also has remarkable properties in the control of bitter rot of apples. The zinc salt showed some promise as a possible protectant of peaches against brown rot.

Because of the large number of possible derivatives of 8-quinolinol, this series lends itself very well to a study of the fungicidal action of the group and the change of toxicity resulting from the substitution of various elements in the parent compound. This paper is an initiation of such a study based on 8-quinolinol and eleven of its derivatives (Table 1).

MATERIALS AND METHODS

The 8-quinolinol and its derivatives were prepared by the Monsanto Chemical Company for experimental use and with the exception of 8-quinolinol sulfate, they were the highest grade obtainable. The 8-quinolinol sulfate was an 8 per cent solution and in a crude form. The ferric dimethyldithiocarbamate used was Fermate produced by E. I. Du Pont de Nemours Company. The chemicals used in making the Bordeaux mixture were Baker and Adamson chemicals produced by General Chemical Company.

Most of these compounds were very insoluble in water. In order to suspend them in water they were made wet with ethyl alcohol and then brought to volume with water. In the case of 8-quinolinol which was

found to be soluble in ethyl alcohol, the material was dissolved in alcohol and then made to volume with water. It remained in solution after the water was added. Since 8-quinolinol sulfate was water soluble, no alcohol was used in preparing the various dilutions of this material. The maximum amount of alcohol was 4 per cent in the dilutions of 1000 parts per million, 0.4 per cent in 100 parts per million, and a proportional amount in other dilutions.

The molecular structure of 8-quinolinol and representatives of the various types of derivatives included in this study are shown in figure 1. The 5, 7 substitution products simply have the H replaced by Cl, Br, or NO_2 in the 5 and 7 positions of the quinolinol ring or rings.

All glassware was cleaned in perchlorate-sulfuric acid cleaning solu-

TABLE 1.—*List of compounds tested, the abbreviations used in the graphs, and the empirical formula of each compound*

Compound	Abbreviation used	Empirical formula
8-quinolinol	8-Q	C_9H_7ON
8-quinolinol sulfate	8-Q SO_4	$(C_9H_7ON)_2H_2SO_4$
Copper 8-quinolinolate	Cu-8	$(C_9H_6ON)_2Cu$
Magnesium 8-quinolinolate	Mg-8	$(C_9H_6ON)_2Mg$
Manganese 8-quinolinolate	Mn-8	$(C_9H_6ON)_2Mn$
Iron 8-quinolinolate	Fe-8	$(C_9H_6ON)_2Fe$
Zinc 8-quinolinolate	Zn-8	$(C_9H_6ON)_2Zn$
5,7-di-bromo 8-quinolinol	di Br	$C_9H_5ONBr_2$
5,7-di-chloro 8-quinolinol	di Cl	$C_9H_5ONCl_2$
5,7-di-nitro 8-quinolinol	di NO_2	$C_9H_5O_5N_3$
Copper 5,7-di-bromo 8-quinolinolate	Cu di Br	$(C_9H_4ONBr_2)_2Cu$
Copper 5,7-di-chloro 8-quinolinolate	Cu di Cl	$(C_9H_4ONCl_2)_2Cu$
Bordeaux mixture	B. M.	
Ferric dimethyldithiocarbamate	Fermate	$(C_3H_6NS_2)_3Fe$

tion as suggested by Morton and Czarnetzky (19) and thoroughly rinsed in tap water and in distilled water.

Two different methods of evaluating fungicides were used. They were the settling tower method and the spore-dilution-drop method as outlined by The American Phytopathological Society Committee on Standardization of Fungicidal Tests (1) except that it was impossible to obtain a water seal since the only moist chambers available had knobs on the tops and could not be inverted. However, the chambers were kept in an air-conditioned room with a temperature of 26° C. ± 1° and a relative humidity of 50 per cent ± 2 per cent so that this lack of water seal should be unimportant under these conditions. Because of the possible effect resulting from the volatilization of these compounds, the chambers and slides were removed to the air-conditioned room after spraying and before the spore-drops were added so that the spores were never in the room in which the spraying was done.

The culture of *Stemphylium sarcinaeforme* (Cav.) Wilts. was obtained from Dr. S. E. A. McCallan of the Boyce Thompson Institute under the

name *Macrosporium sarcinaeforme,* but this was later changed by McCallan (13).

Each treatment was applied to four slides with three drops on each slide. These slides were incubated in one moist chamber together with one untreated slide which was included in order that the volatile effect of the compound could be manifested. One chamber containing four untreated slides was used as a daily check on the normal germination of the spores with no fungicide present. The spores were counted after 18–20 hours' incubation at 26° C.
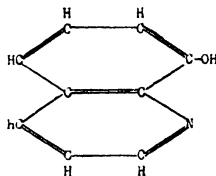
A. 8-QUINOLINOL
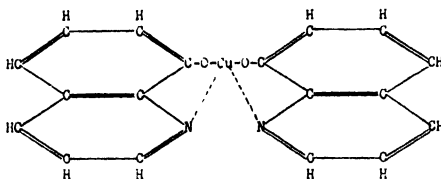
B. Metal salt of 8-QUINOLINOL
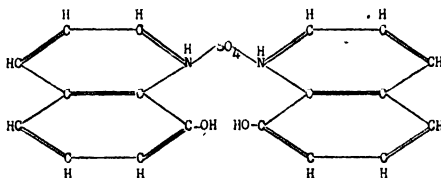
C. 8-QUINOLINOL SULFATE

FIG. 1. Structural formulae of representative types of 8-quinolinol compounds.

The method used for determining whether the action of these compounds, which at some point permitted no germination, was fungicidal or fungistatic was similar to that outlined by McCallan and Wellman (14). The compounds which were not completely water soluble were suspended in a flask, stoppered, and shaken for 20 hours on a reciprocal shaker with a 2½-inch stroke at 100 cycles per minute. This suspension was then centrifuged at 870 times gravity for 5 minutes and the supernatant liquid was decanted and used in the same way as the completely water soluble materials. Spores were introduced into these solutions and tests were made by the spore-dilution-drop method to make certain that the solutions were concentrated enough to permit no germination. The remainder of the spore suspension was allowed to remain in a test tube. If no germination occurred, then the suspension was centrifuged and the spores were washed three

times with distilled water. The spores were then brought back to the original volume with distilled water and tested again by the spore-dilution-drop method. Lack of germinated spores at this point indicated that the material was fungicidal; whereas, the presence of germinated spores indicated that the material was fungistatic.

The results of the tests by the settling tower method are represented in figures 2 and 3. The values are an average of 24 counts of 50 spores so that each value is actually the percentage germination based on 1200 spores. These graphs are plotted on logarithmic-probability graph paper.

The results of the tests by the spore-dilution-drop method are presented in figures 4 and 5 in the same manner as figures 2 and 3 except that the concentrations are in parts per million as prepared before the drops were put on the slides.

All of the compounds were tested over essentially the same range of concentrations from 0.5 to 1000 parts per million and from 0.01 to 35 micrograms per square centimeter. Higher concentrations were impractical because of the difficulty of distinguishing the spores from the insoluble material on the slides.

In the fungicidal versus fungistatic tests, it was found that 8-quinolinol, the copper salt, the halogenated derivatives and their copper salts, and 8-quinolinol sulfate were fungicidal under the conditions of these tests. This is not in agreement with the findings of McCallan and Wellman (14) on 8-quinolinol sulfate. However, since the 8-quinolinol sulfate used in these tests was in a crude form, it is possible that other substances may have influenced these results.

The compounds showing vapor toxicity were 8-quinolinol sulfate, 8-quinolinol, the magnesium salt, the manganese salt, the zinc salt, the dichloro derivative, and ferric dimethyldithiocarbamate. This effect was observed only in a qualitative manner since a quantitative method of measuring vapor toxicity is beyond the scope of this study. These results are in agreement with those of Lebduska and Pidra (11). They reported that the vapors of 8-quinolinol inhibited the growth of *Staphylococcus aureus* and *Bacillus coli* in Petri dishes containing one gram of the compound and held for 24 hours.

The data presented do not seem to follow the usual pattern of toxicity reactions. All the metal salts of 8-quinolinol (except the copper salt) generally reduced germination with an increase in concentration only to a certain point (figs. 2 and 3). This was then followed by an increase in germination as the concentration was increased further. The same tendency was observed for these compounds in the results represented in figure 4. However, the magnesium salt reversed itself after the concentration reached 180 parts per million and a further increase in concentration resulted in a

decrease in germination. The 8-quinolinol and 8-quinolinol sulfate followed this same trend in the spore-dilution-drop method but not in the settling tower method. McCallan, *et al.* (15) have reported that certain inorganic compounds exhibit a departure from a linear dosage-response curve. Dimond *et al.* (3) also reported a dosage-response curve that was not a straight line for laboratory tests on tetramethyl thiuramdisulfide. However, Horsfall (8) implies that when such trends are observed, one should first examine his methods carefully. In the case of the data presented the same tendency was observed in each of duplicate tests employing two different methods and with each test conducted on different days. The fact that repeated tests in which Bordeaux mixture and the copper salt were used gave very consistent normal results further reduces the probability of these trends being the result of some discrepancy in the methods employed.

Limited field observations have shown a similar trend in the action of the zinc salt when sprayed on peaches as a protectant against brown rot. One quarter pound per 100 gal. of water was more effective than one half pound, which in turn was more effective than one pound in controlling the disease. None of the theories at present seem to adequately explain this type of reaction. It may be that the particles formed larger aggregates as the concentration was increased, and, as a result, there may have been less surface exposed for solution and subsequent reaction with the spores to reduce germination. Gortner (6, p. 186) has presented a table showing the tremendous decrease in surface area for a specific weight of a substance as the particle size is increased. These materials are only very slightly soluble in water and it is possible that with a decrease in exposed surface area through aggregation not so much material would be dissolved in concentrated suspensions and thus brought into reaction with the spore as in the lower concentrations. Another possibility is that suggested by Dimond *et al.* (3). They pointed out the possibility of a change in reaction phase of a material by dissociation or association so that its toxicity is markedly different from the original molecule. If this is true then the toxicity curve should be expected to follow the association-dissociation curve of the same compound.

A third possible factor that has been suggested as having some importance in this reaction is the permeability of the spore at various concentrations of the salts tested. Jacobs (10) cites the work of Osterhout showing that certain compounds may cause a definite decrease in permeability of plant tissue at low concentrations but that an increase in concentration may then cause an increase in permeability to the point where death of the tissue would be expected. If reduction in germination is proportional to the increase of permeability, then the findings of Osterhout would not explain the reactions reported here. This does not, however, eliminate the possible importance of permeability since the 8-quinolinols may affect permeability in a different manner than the compounds used by Osterhout.

The fact that the compounds showing vapor toxicity are the ones pro-

ducing the two-peaked dosage-response curve may be a significant factor in explaining these reactions. Further investigation is needed to show if this connection is significant and the possible relationship if it is significant.

The halogenated derivatives of 8-quinolinol as shown in figures 2, 3, 4, and 5 produced the same general type of reaction as Bordeaux mixture, ferric dimethyldithiocarbamate, and copper 8-quinolinolate (with the exception of the two di-bromo derivatives) by the settling tower method. Copper 5, 7-dibromo 8-quinolinolate did not reduce germination to less than 60 per cent. The halogenated derivatives were much less effective than 8-quinolinol or copper 8-quinolinolate in reducing the germination of spores. Conversion of parts per million to molar concentration failed to eliminate this difference in effect. Thus it is apparent that the substitution of chlorine and bromine for hydrogen in the 5 and 7 positions definitely decreased the ability of the compounds to reduce spore germination of *Stemphylium sarcinaeforme*. The same was true of the 5, 7-di-nitro 8-quinolinol. There was no reduction in germination by this compound at any concentration tested.

This group of compounds responds in a manner different from the phenols as reported by Woodward *et al.* (22) when halogen atoms were substituted into these compounds. They found that the toxicity to the test organisms employed was multiplied several times by such substitutions into the phenols.

The loss of toxicity of 8-quinolinol when substitutions of the nitro group are made at the 5 and 7 positions suggests that toxicity may be the result of a reaction of 8-quinolinol with certain nitrogenous materials vital to the organism. If this reaction has occurred before the compound comes in contact with the organism, such as in the di-nitro derivative, no toxicity would be evident. On the other hand, the substitution of nitro groups or halogens may cause a shift in the internal structure of the molecule in some manner so as to change its reaction.

Copper 8-quinolinolate was found to be considerably more effective than any other material tested by the spore-dilution-drop method. The 8-quinolinol sulfate was slightly more effective in the tests by the settling tower method, but even by this method the concentration of complete inhibition of germination was the same for both materials. Either material was more effective than Bordeaux mixture. The difference is even greater than is indicated in figure 2 because of the fact that the concentration of Bordeaux mixture is based on the deposit of copper only; whereas, the concentration of the other two compounds is based on the deposit of the total material. On the basis of parts per million of copper, the concentration of the copper 8-quinolinolate which will permit no germination is only 1.4, and the concentration had to be reduced to 0.17 parts per million to permit 100 per cent germination.

McCallan and Wilcoxon (16) in comparing several copper fungicides with Bordeaux mixture concluded that fifty years of experimentation with

copper compounds have yielded none equal to Bordeaux mixture. They also stated that apparently no copper compound has yet been developed which has combined high fungicidal values with low phytocidal properties.
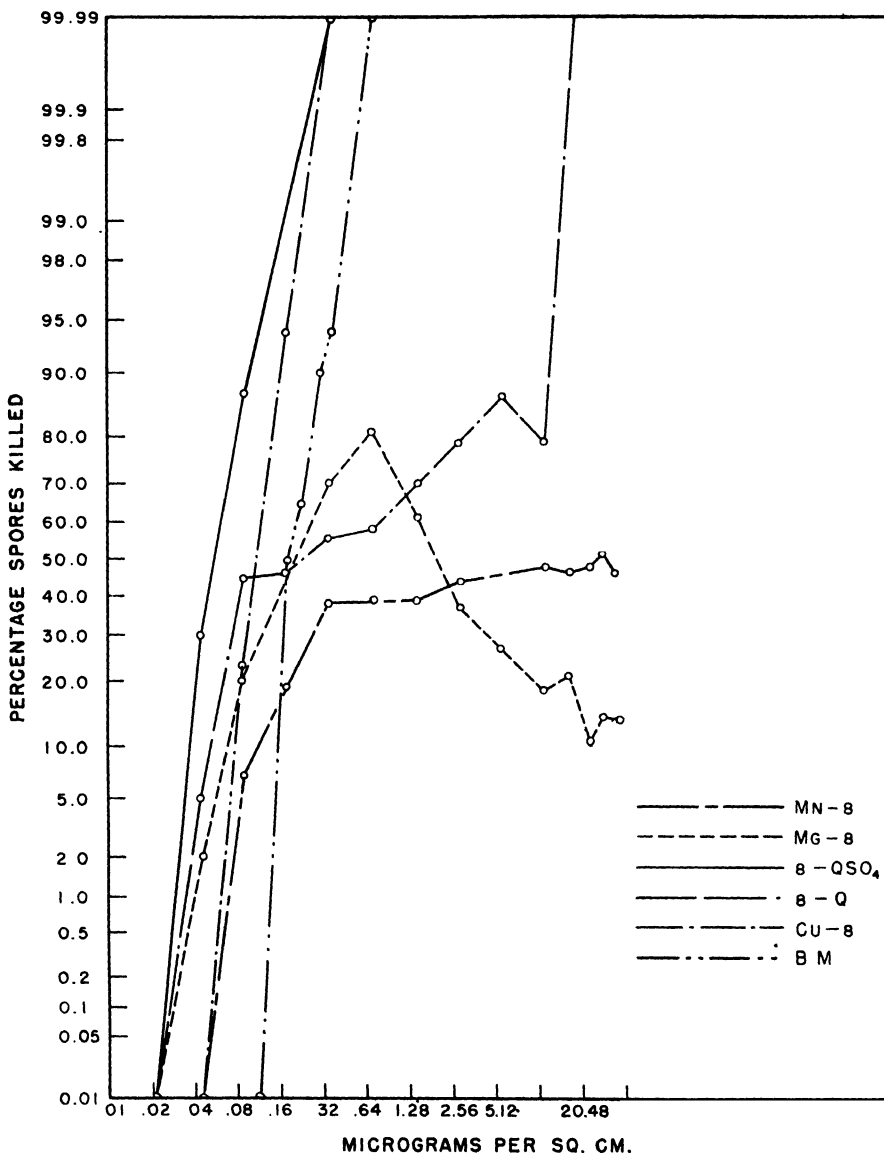
FIG. 2. Dosage-response curve of *Stemphylium sarcinaeforme* by the settling tower method.

It is possible that copper 8-quinolinolate may eventually compare favorably with Bordeaux mixture as a fungicide. In these laboratory studies it has shown a definite superiority over Bordeaux mixture in fungicidal activity. Various field tests in Illinois in 1946 have indicated that it has no notice-

able harmful effect on apples, grapes, cherries, or tomatoes. No serious injury was observed when peaches were sprayed with concentrations of ¼ lb. per 100 gal. of water or less. In greenhouse tests no injury was observed on beans when they were sprayed three times at weekly intervals
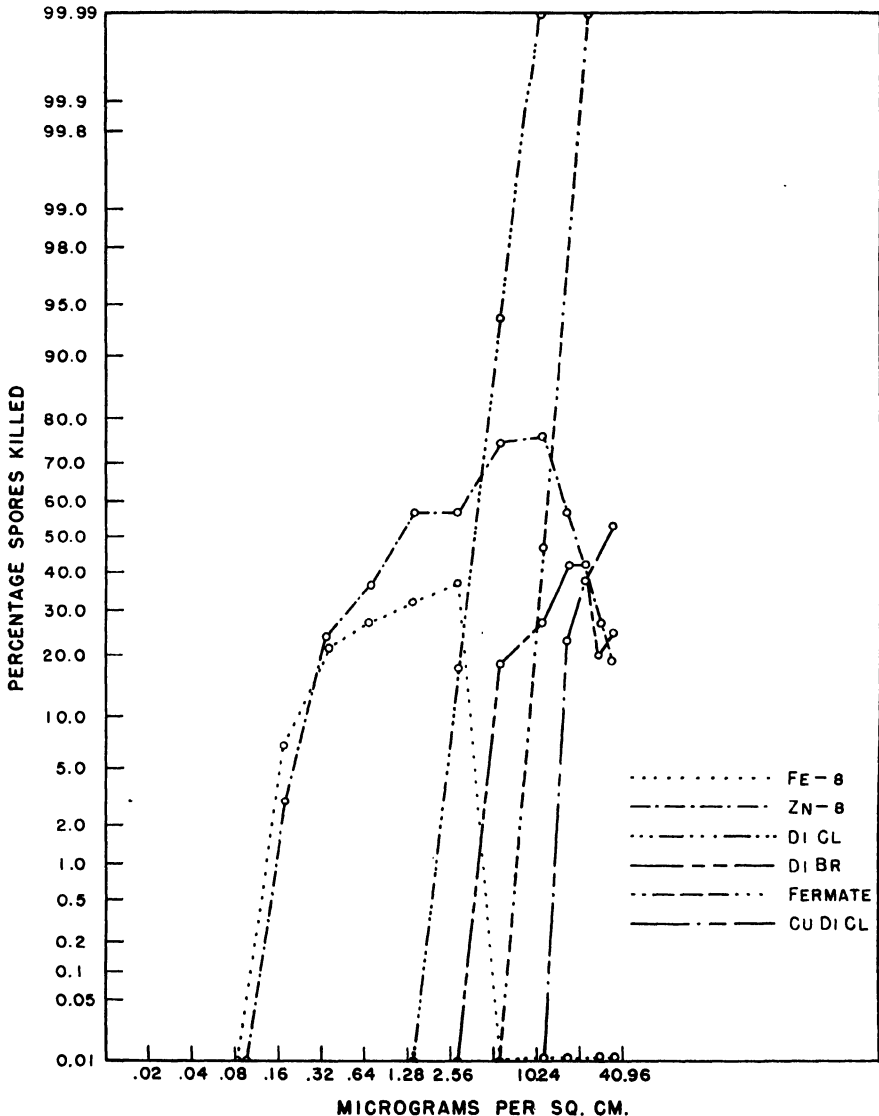
FIG. 3.   Continuation of figure 2.

with concentrations as high as 4 lb. per 100 gal. Furthermore, this compound is only very slightly water soluble and will adhere to foliage for several weeks even when exposed to heavy rain. No injurious effect to man has been observed.

The pronounced variations of these compounds in their ability to affect

the fungi tested is particularly noteworthy when one considers the chemical similarity of the compounds.   Zentmeyer (23 and 24) has advanced the idea that the toxic action of 8-quinolinol may be due to its precipitation of the heavy metals essential to the test organism.   By adding zinc to
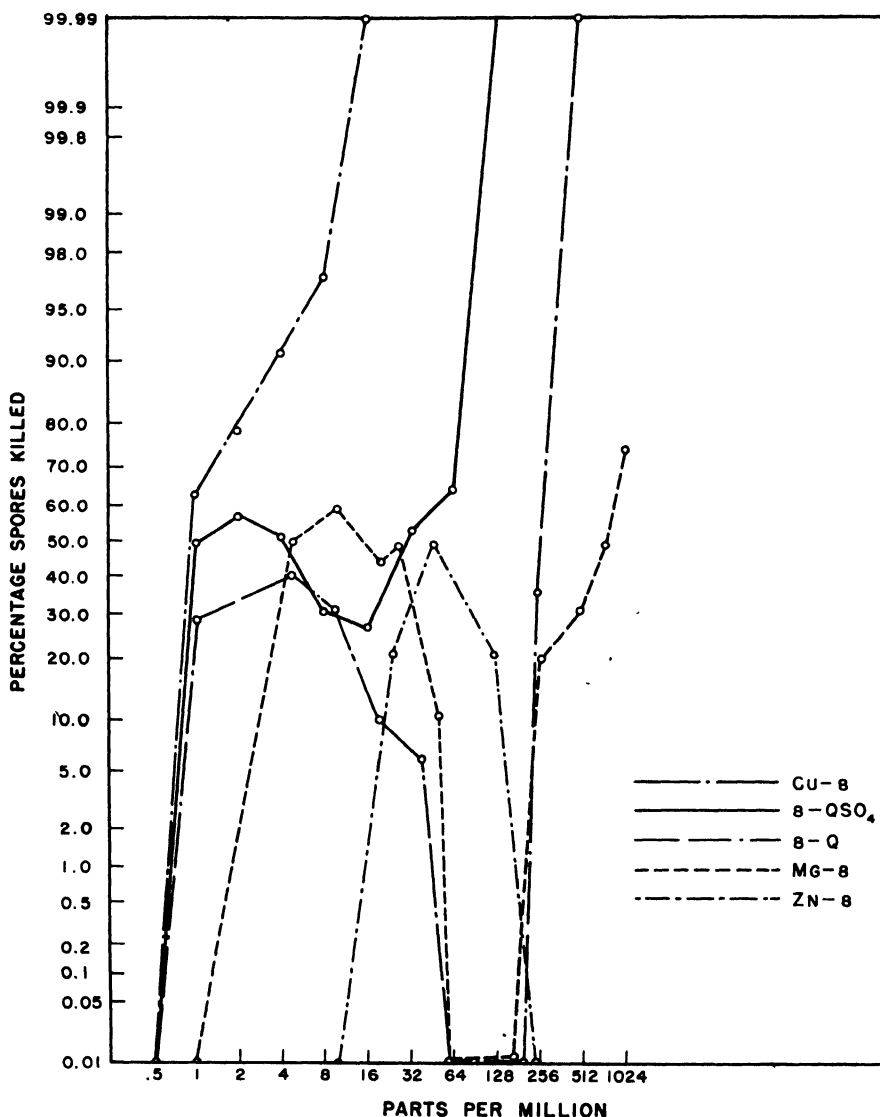
FIG. 4.   Dosage-response curve of *Stemphylium sarcinaeforme* by the spore-dilution-drop method.

8-quinolinol he was able to nullify the effect of the 8-quinolinol.   This theory would not explain the superiority of copper 8-quinolinolate over 8-quinolinol as observed in these experiments, since the 8-quinolinol would ,already be saturated with copper.   Furthermore, 8-quinolinol is less efficient than the magnesium, manganese, and zinc salts in some parts of their

dosage-response curves (Fig. 4). According to Zentmeyer's theory it should be expected that 8-quinolinol would always be more efficient than either of its heavy metal salts.

For compounds showing the type of reactions reported here, it is ap-
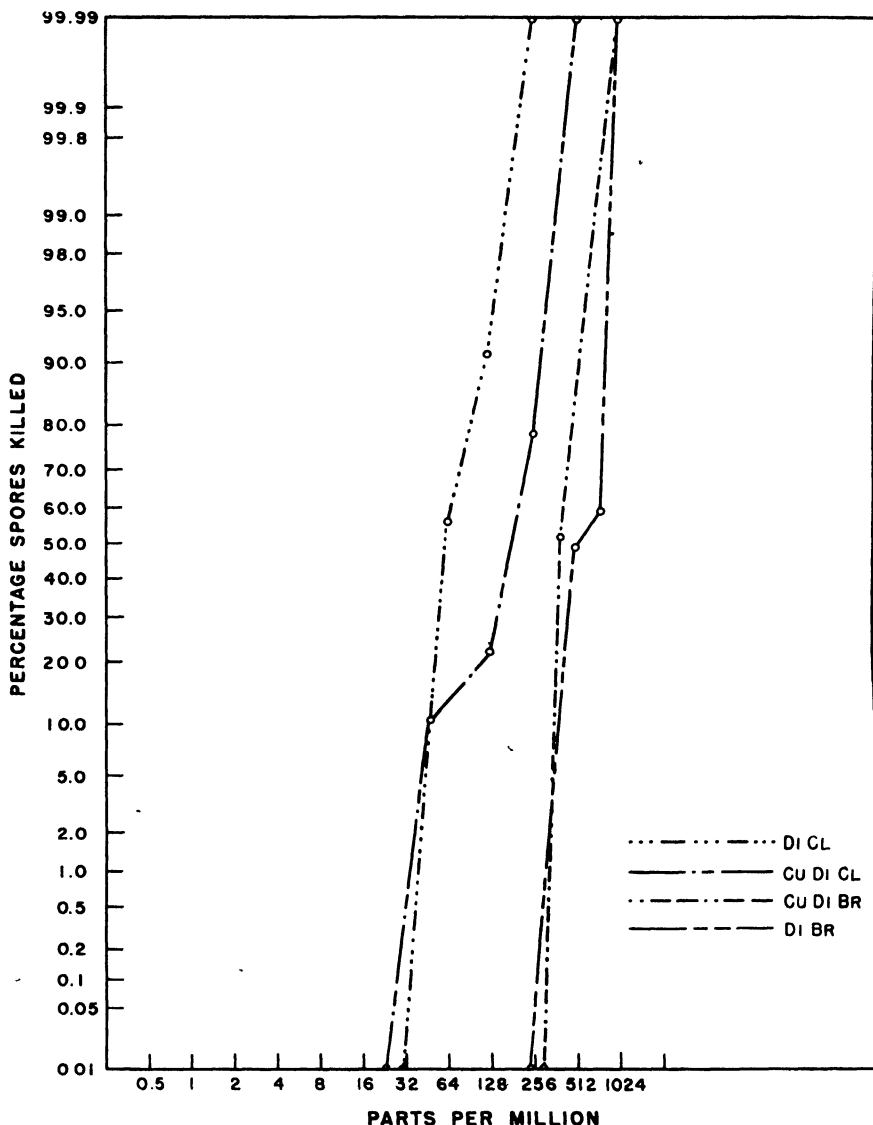
FIG. 5. Continuation of figure 4.

parent that the L.D. 50 is not a suitable index of comparison. McCallan *et al.* (15) stated that for such compounds the L.D. 95 is a much better index of comparison. If the compound shows any promise as a fungicide, it is suggested that a determination of the entire range of dosage-response and even beyond the point of L.D. 100 is desirable in order to make certain

whether there is a double effect as observed in this group of compounds. This point should not be difficult to obtain in laboratory tests with a powerful fungicide.

SUMMARY AND CONCLUSIONS

Fungicidal tests of 8-quinolinol and eleven of its derivatives by two different methods were studied. The results were tabulated and a wide range of activity for these chemically related compounds was observed. All of the metal salts of 8-quinolinol except the copper salt produced a two-peaked curve in their effect on the germination of spores of *Stemphylium sarcinaeforme*.

The substitution of chlorine or bromine atoms for hydrogen in the 5 and 7 positions was found to reduce the toxicity of 8-quinolinol. This reduction in toxicity was also observed in the copper salts of these derivatives. The substitution of nitro groups in the 5 and 7 positions eliminated the toxic action of 8-quinolinol entirely.

Copper 8-quinolinoate was found to be more effective than Bordeaux mixture in reducing the germination of spores of *Stemphylium sarcinaeforme*.

The conversion of concentrations from parts per million to molarity did not eliminate the wide variations in concentrations of compounds with different molecular weights necessary to reduce or prevent the germination of spores of *Stemphylium sarcinaeforme*.

The compounds of this group which at some point permitted no germination of spores of *Stemphylium sarcinaeforme* were found to be fungicidal rather than fungistatic.

From the results of these studies the following conclusions may be made:

1. These compounds did not produce a straight line dosage-response curve as most other compounds do.

2. The fact that not all compounds followed a straight line dosage-response curve makes the use of L.D. 50 a doubtful index for comparing one fungicide with another, unless the entire dosage-response curve from L.D. 0 to L.D. 100 has been determined.

3. The precipitation of the heavy metals by 8-quinolinol so that they are no longer available to the test organism does not explain completely the toxic action of 8-quinolinol and its derivatives tested in these experiments.

4. Substitution of chlorine and bromine atoms for hydrogen atoms in the 5 and 7 positions of 8-quinolinol did not result in an increase in the toxicity of the compound as has been reported for halogen substitutions in some phenol compounds.

5. Of the compounds tested copper 8-quinolinolate proved to be the most efficient fungicide, and it was found to have many properties desirable in a good plant protectant.

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    URBANA, ILLINOIS.

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New Hosts for Botrytis Elliptica.[1]—*Botrytis elliptica* (Berk.) Cke., the cause of a well-known leaf rot and spot of *Lilium* spp., was observed on two uncommon non-lily hosts in western Washington in 1947. Because of the potential threat to the expanding bulb and corm industry in the Pacific Northwest it is deemed important to briefly point out these cases of increase in known host range of this species. The newly noted hosts are autumn crocus (*Colchicum autumnale* L.) which was found infected near Olympia and the following varieties of *Gladiolus* spp. growing near Auburn: Rosa van Lima, Greta Garbo, Bach, Anna Mae, Cave-Cole and Jeannie.

On *Colchicum* the organism caused severe spotting of the older leaves and leaf-tip killing of the younger ones. The spots were circular to elon-
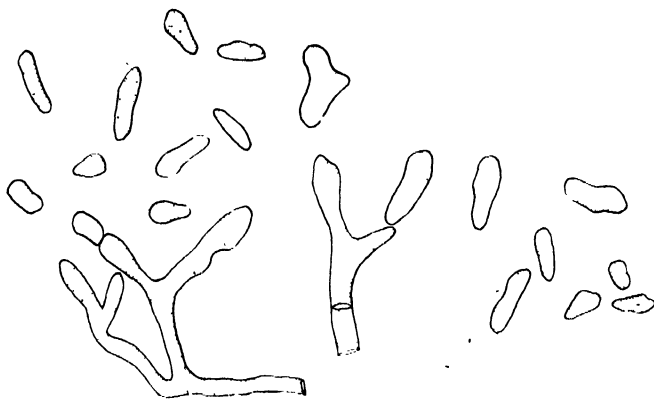
FIG. 1. Camera lucida drawing ×700, showing spores of *Botrytis elliptica* and apical portions of conidiophores with typical branching.

gate, brown to yellow-brown with pale centers. Grayish-black spore masses were found on all affected parts. The numerous conidiophores were at first hyaline with pale brown walls, later becoming olivaceous-brown. They bore clusters of elliptical to elongate, hyaline, later pale brown conidia at their tips (Fig. 1). The conidia measured 17.5–35.5 × 10–14 μ (mean size 28.0 × 10.5 μ).

On *Gladiolus* spp. the same organism caused a very severe spotting on seedling stock, often completely killing the young leaves. The spots were numerous, small (2–5 mm. diam.), circular to slightly elongate, red to reddish-brown with slightly raised centers (Fig. 2). No fruiting structures were observed on the leaves in the field, but isolations showed the typical hyaline, elliptical conidia of *Botrytis elliptica*. The conidia measured 20–34 × 10–16 μ (mean size 25 × 11 μ). Inoculation experiments using healthy *Gladiolus* leaves produced the same symptoms described above.

[1] Published as Scientific Paper No. 762, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

The inoculated leaves were incubated in a moist chamber at 20° C. Early symptoms appeared after five days.

The isolates from these new hosts agree very closely with published descriptions of *Botrytis elliptica*. Moore[2] states that clusters of elliptical, hyaline, later sometimes pale brown conidia measuring 20–25 × 14–16.5 μ (mean 24 × 16 μ) are borne on numerous branched conidiospores, 1–3 mm. long. Westerdijk and van Beyma[3] give the spore sizes as 16–34 ×

FIG. 2. Lesions produced by *Botrytis elliptica* on seedling stock of *Gladiolus* sp., variety Greta Garbo. × ½ nat. size.

10–24 μ.—NEIL ALLAN MACLEAN, Department of Plant Pathology, State College of Washington, Pullman, Washington.

Phytophthora Stem Canker of Sesame in Peru.[1]—Late in 1946 twenty-four selections of Sesame, *Sesamum indicum* L., were received at the Estacion Experimental Agricola de Tingo Maria from Centro Nacional de Agronomia, El Salvador. The first trial of these varieties was therefore during the wet season. Just prior to maturity of the fruits in late February, 1947, wilted plants were observed in the lower and poorer drained portions of the plantings and on closer examination extensive stem cankers, similar to that shown in figure 1, were found on a majority of the plants

[2] Moore, W. C. Diseases of bulbs. Ministry of Agriculture and Fisheries, Great Britain, Bul. 117: 42–46. 1939.

[3] Westerdijk, J., and F. H. van Beyma. Die Botrytis-Krankheiten der Blumenzwiebelgewachse und der Paeonie. Meded. Phytopath. Lab. Willie Comelin Scholten 12: 1–27. 1928.

of all varieties.   These cankers were not localized in any part of the plant but were general from the collar to the tip of the growing point.   Girdling infections at the collar resulted in complete wilting of the plant while infections on the upper portions usually resulted only in the loss of laterals actually involved in the infected zone.   Apparently the leaves or young lateral branches served as the infection court, from which the pathogen advanced to the main stem and caused the stem cankers.   These cankers were reddish brown with irregular advancing edges, and they spread vertically

FIG. 1. Phytophthora stem canker of sesame, showing wilting.

far more rapidly than horizontally.   Dissection of the cankers indicated that the pathogen was primarily an invader of cambium and phloem tissue. Some infection of the fruits was also observed.

Pure culture isolations from the advancing margins of cankered areas yielded almost entirely a *Phytophthora* sp. characterized by sporangia 51.0– 37.4 × 23.8–27.2 μ, averaging 39.7 × 25.5 μ, produced on branching sporangiophores, and oogonia averaging 25.5 μ with paragynous antheridia.   This *Phytophthora* sp. is apparently referable to *P. cactorum* (L. and C.) Schroet.

All Sesame varieties under trial were inoculated with the *Phytophthora* by placing, in a cut extending through the bark, phloem, and cambium tissue, a small amount of mycelium from a corn meal agar culture. Ten days after inoculation extensive cankers had developed from many of the inoculations. Differences in susceptibility of the various Sesame varieties and the greater susceptibility of most of the varieties on excessively wet sites as compared to drier sites are shown in table 1.

TABLE 1.—*Length of cankers, in centimeters, ten days after inoculations on varieties of sesame, Sesamum indicum, showing differences in susceptibility of varieties on wet and dry sites*[a]

Variety[b]	TM. No.	Wet site	Dry site
Brazilian	1452	15.30[c]	2.40
Natural Indian White	1453	14.70	5.20
Indian White No. 2	1455	9.30	1.50
Indian No. 1	1456	2.30	0.00
Indian FAQ	1457	13.80	0.40
Columbian	1458	7.80	0.20
Indian Natural	1459	10.30	2.30
Brazilian White	1460	7.40	5.20
Indian No. 2	1461	15.20	3.30
Guatemala	1462	11.10	0.20
Nicaraguan	1463	12.20	1.60
Bombay White	1464	8.20	1.80
Mexican White	1466	11.20	6.50
Java Black	1467	11.70	2.10
Mexican	1468	10.90	6.20
Nicaraguan White	1469	12.60	10.60
Syrian Reddish	1470	1.80	2.10
Chinese Yellow	1471	3.60	2.90
Turquestan 2685	1472	12.80	8.10
Turquestan 2683	1474	12.30	8.20
San Salvador	1475	10.70	4.50

[a] Site differences for the last nine varieties were less pronounced than for the first twelve varieties.

[b] Three varieties, Indian White No. 1, China White, and Indian White were early maturing and could not be included in the test.

[c] Inoculations took 100 per cent, each figure represents average of 5 plants inoculated.

At present the disease, while serious, does not appear to be a limiting factor to the growing of Sesame in this region. The greater susceptibility of plants on wet sites indicates dry season crops and wet season crops on well drained sites should not be too adversely affected by the disease.

In so far as the writers have been able to determine no similar disease of Sesame, caused by a *Phytophthora,* has been reported from Peru or elsewhere; however a root rot of this species in Santo Domingo caused by an unidentified *Phytophthora* has been reported[2] and might possibly be a manifestation of the same disease.—BOWEN S. CRANDALL and JAVIER DIEGUEZ C. respectively, Pathologist, Office of Foreign Agricultural Relations, U. S. Department of Agriculture and Chief, Dept. of Plant Pathology and Entomology; and Assistant Chief, Department of Plant Pathology and Entomology, Estacion Central de Colonizacion en Tingo Maria.

[2] Ciferri, R. Phytopathological Survey of Santo Domingo, 1925–1929. Puerto Rico Dept. Agr. Jour. 14: 5–44. 1930.

A Technique for Growing Citrus Seedlings under Aseptic Conditions of Culture.—The object of devising the method described below was to grow in pure culture citrus seedlings to be used for damping-off studies with *Rhizoctonia solani* Kühn.
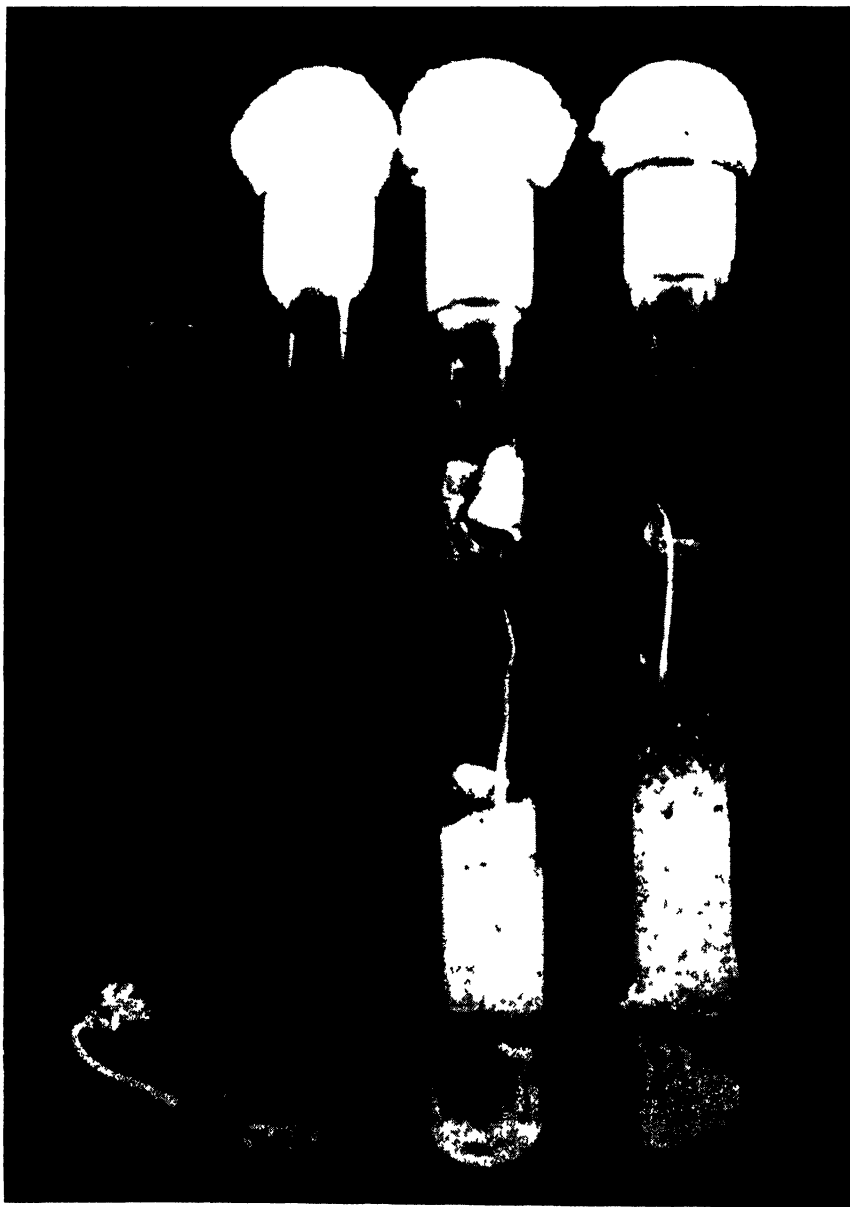
FIG. 1. Method of growing citrus seedlings under aseptic conditions of culture for inoculation with soil fungi: A. Glass test tube $6 \times \frac{3}{4}$ inches, with cotton wick passing through hole in bottom, and a larger tube of 8×1 inch dimensions containing a short glass rod. B. Healthy citrus seedling growing in quartz sand saturated with sterile nutrient solution. C. Damped off citrus seedling after inoculation with *Rhizoctonia solani.*

With favorable temperature and soil moisture, citrus seeds usually take from 15 to 30 days to germinate. While the seeds can be germinated on sterile agar in test tubes, the procedure is less efficient than the one here described because of the following handicaps: 1. The surface of the agar in a test tube has to be broken before planting the seed; otherwise, after 3 to 4 days' incubation at 31°–33° C., it will become too hard for the young radicle to penetrate. 2. The seed does not obtain enough moisture from the agar to germinate within a normal time, thus delaying the germination unnecessarily.   3. After 15–20 days of incubation at 31°–33° C., the agar has shriveled so much that the young roots are found to hang along the walls of the test tube, where because of lack of moisture they dry and curl.

In order to overcome these handicaps and to secure seedlings under sterile conditions, agar was replaced with sand. With a blow-torch a hole about ⅛ inch in diameter was made in the bottom of an ordinary glass test tube of 6 × ¾-inch dimensions. A cotton-thread wick about 1½ inch long and ⅛ inch in diameter, and with a knot in one end, was inserted through the hole so that the knot rested within the tube (Fig. 1, A). The test tube was filled with previously washed and dried fine quartz sand to a depth of 1¼ inches. The small test tube thus equipped was placed carefully into a larger glass test tube of 8 × 1-inch dimensions and having a one-inch piece of a glass rod and about 15 ml. of nutrient culture solution in the bottom. The larger test tube was plugged with cotton and autoclaved 20 min. at 15 lb. pressure.

After the contents of the tube are cool, one seed per test tube was planted aseptically and incubated at 31°–33° C.[1] The sand saturated with nutrient culture solution not only supplied enough moisture for germination of the seed but also fed the young roots for at least two months during which period inoculation experiments could easily be concluded (Fig. 1, B and C).

This device may also be employed for other types of seeds that require a relatively long time to germinate.

In this apparatus with the nutrient in the pH range, 4.85 to 8.85, and the incubation temperature at 25° C., a virulent strain of *Rhizoctonia solani* will damp-off sweet-orange seedlings within 3 to 5 days after inoculation.

The writer wishes to express his appreciation to Dr. L. J. Klotz for his guidance in the preparation of this note.—INAM U. KHAN, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California.

A Method of Testing Beans for Resistance to Bacterial Blights.—A propram of breeding bush snap beans (*Phaseolus vulgaris*) with resistance to the common blight (*Xanthomonas phaseoli* [E. F. Sm.] Dowson) and the halo blight (*Pseudomonas medicaginis* var. *phaseolicola* [Burkh.] Stapp and Kotte) has been proceeding at the U. S. Regional Vegetable Breeding Laboratory since 1938. It has resulted in several new garden types, including Fullgreen No. 1 and No. 2, B1788, and B2051, now on trial with seedsmen.

[1] Fawcett, H. S. Temperature experiments in germinating orange seeds. California Citrograph 14: 515. 1929.
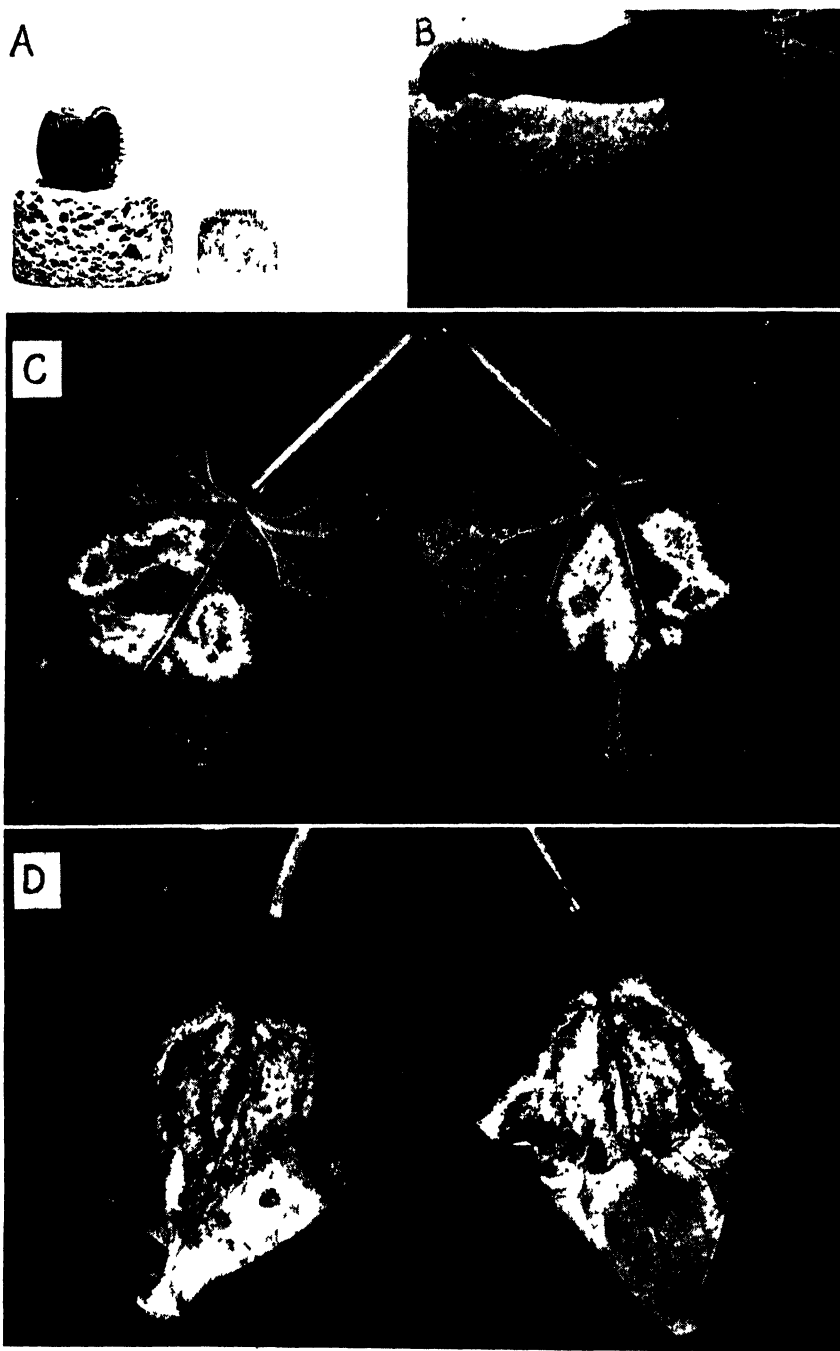
' Fig. 1. Bacterial inoculation of bean leaves. A. Multiple needle inoculators and cellulose sponge. B. Inoculation of bean primary leaf. C. A resistant reaction to *Xanthomonas phaseoli* 14 days after use of the multiple needle inoculator. D. A susceptible reaction 14 days after use of the multiple needle inoculator.

During this period we have tried many different methods of inoculating bean plants to differentiate the various grades of resistance or susceptibility. These methods have included high-pressure and low-pressure sprays, seed-soaking, seed injection and stem injection with hypodermic needles, and leaf abrasion. All were discarded in 1942, and in all tests since then a new technique has been employed, which we call the "multiple needle, primary leaf inoculation."

The multiple needle inoculator was constructed with 90 No. 0 insect mounting pins with their head ends imbedded in a block of sealing wax (Fig. 1, A). The primary leaf is placed upon a rubber or cellulose sponge saturated with bacterial suspension (Fig. 1, B) and two strokes are used in passing the inoculator through the leaf into the sponge. The first stroke forces the leaf into close contact with the wet sponge and the second stroke draws additional inoculum into the leaf.

Plants are grown in pots and inoculated on about the 14th day, or when the first trifoliate leaf is beginning to open. Readings are made 12 to .14 days after inoculation, and are based on the amount of breakdown of tissues extending from the inoculated area. In order to produce a more uniform development of the primary leaves, all terminal and axillary growth is kept pruned away.

The method offers two principal advantages: (1) There are no escapes; and (2) it provides a reaction area that can be measured quantitatively (Fig. 1, C, D). Also it has emphasized two important environmental influences on bean reaction to these blights: (1) At high temperatures (above 80° F.) all varieties tend to appear susceptible; and (2) abnormally weak plants or plants attacked by root rots (*Rhizoctonia, Fusarium*) tend to have an increased susceptibility.

In a highly blight-tolerant line, a weak plant may be rather susceptible; likewise in a very susceptible line, an exceptionally vigorous plant may appear tolerant. This emphasizes in another way the importance of high-quality seed. The beneficial effects of western-grown bean seed may not be due entirely to its freedom from blight. Home-grown seed in many districts is apt to be weak, improperly matured, poorly graded, and in general will give a higher percentage of weak plants that are hyper-susceptible to blights.

The method described can be adapted to other bacterial diseases of plants. —C. F. ANDRUS, U. S. Department of Agriculture, Regional Vegetable Breeding Laboratory, Charleston, South Carolina.

OXYGEN AND CARBON DIOXIDE RELATIONS OF FUSARIUM OXYSPORUM SCHLECHT. AND FUSARIUM EUMARTII CARP.[1]

JOHN P. HOLLIS[2]

The effects of different oxygen and carbon dioxide concentrations upon fungus growth in soil have been of little interest to plant pathologists as a whole. Pure culture studies (2) have shown that species in the genus *Fusarium*, like other fungi, are tolerant of a wide range of oxygen and carbon dioxide concentrations. This fact has retarded recognition of the soil atmosphere, with its variable constitution in different soils, as playing a determinative role in the etiology of root diseases. Particularly is this true for diseases caused by species of *Fusarium*.

An attempt has been made in the present study to explore the possible effects of varied concentrations of the metabolic gases upon the growth, survival, and morphology of two species of *Fusarium* in pure culture and in soil apart from their hosts. The results are comparative in point of method and in the fact that *F. oxysporum* Schlecht. and *F. eumartii* Carp. are related taxonomically, physiologically, and pathologically. The purpose of this paper is to draw attention to certain physiological differences existing between these organisms. These differences have been recorded and evaluated in terms of survival, morphology, and relative growth rates under the various experimental conditions.

Physiological studies have been made of several important pathogens with regard to oxygen (6, 8, 12, 19, 20), carbon dioxide (2, 4, 6, 13), growth in soil (1, 7, 9, 11, 24), and forced aeration (5, 7, 10, 18).

The economic importance of diseases caused by *Ophiobolus graminis* Sacc., *Actinomyces scabies* (Thaxt.) Gussow and *Phymatotrichum omnivorum* (Shear) Duggar has provoked extensive studies which include the soil atmosphere as a possible factor controlling the extent of these fungi in particular soils. Here, as with other important soil pathogens, the role of soil moisture has been of primary interest. When air content has been considered, it is generally as a function of moisture content and soil texture.

Soil distribution studies (14, 15, 16, 17) of certain species in the genus *Fusarium* have been of aid in formulating regional patterns of their ecology in limited areas of the globe, but knowledge of distribution of these organisms in the United States has arisen mainly from reports of diseased plants and isolations from soil. Specifically we know of no reasons for the natural distribution of *F. oxysporum* Schlecht. and *F. eumartii* Carp. in the United States. It was hoped that a study of oxygen and carbon dioxide relations might yield information on this point.

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<div style="text-align:center">EXPERIMENTAL METHODS</div>

Cultures of *Fusarium oxysporum* 293 and *F. eumartii* 257 obtained from the Department of Plant Pathology, University of Nebraska, were subjected to lowered oxygen tensions and increased carbon dioxide tensions in pure culture. Petri dishes, 100 mm. in diameter, containing measured quantities of 2 per cent potato-dextrose agar were inoculated with discs, 6 mm. in diameter, of actively growing mycelium. The reaction of the medium was standardized between pH 6.6 and 6.8. Growth measurements were recorded as the average of two diameters of the fungus colony.

Lowered oxygen tensions were obtained by using the alkaline-pyrogallol method prescribed by Buchner (3). The following equation was devised to determine quantities of alkali and acid required to establish different tensions and accounts approximately for the change in volume of chamber when alkaline pyrogallol solution is added.

$$X = \frac{(V - D)}{11}$$

where:

      V = chamber volume
      D = displacement of air by cultures
      X = ml. 10 per cent KOH
      $\frac{X}{10}$ = gm. pyrogallic acid

The initial tensions thus determined theoretically were checked manometrically and recorded in terms of mm. Hg. Gas analysis tests and amounts of nitrogen required to bring the chambers up to atmospheric pressure at the end of an experiment were carried out in some cases where the organisms ceased growth.

Limitations of this closed system method are obvious in that the supply of oxygen is gradually decreased during a test; however, it is considered to approximate certain natural conditions found in the soil more closely than the constant aeration method.

Different carbon dioxide levels were established by collecting measured quantities of the gas over water and introducing it into evacuated chambers. Exact concentrations were determined by analysis of duplicate samples and are expressed below as percentages.

Growth of the organisms in soil was studied by using mixtures of varied composition including sand, sand plus composted soil, composted soil, and field soil in glass tubes. Attempts were made to control the air content by varying composition, degree of packing, moisture content, and forced aeration. Two types of glass tubes were used. The smaller tubes, 23 cm. by 1.6 cm. internal diameter, were plugged at the lower end with glass wool and at the upper end with cotton. The tubes containing equal, weighted quantities of soil were autoclaved for 30 minutes at 15 pounds pressure, then placed upright in sterile water or dilute sucrose solution and saturated by capillary rise. To insure uniform packing and moisture-holding capacity the tubes

were dropped from a standardized height before and after saturation. Even distribution of sand particles was secured by rapidly rotating the tubes. In order to obtain the desired moisture content, it was necessary to aspirate dry air through the tubes or to place them in a drying oven until the desired weight of tube and soil was obtained. After inoculation the tubes were incubated by placing them upright in a large moist chamber which contained an air inlet plugged loosely with cotton.

The extent of growth between soil and tube was measured with a dissecting microscope and a millimeter rule and was recorded at weekly intervals. The central two-thirds of the soil cylinder was considered most representative of a given moisture level because of slight drying at the top and moisture accumulation at the bottom of the tube. Isolations were made from the center of the soil cortex.

Large tubes of 2.2 cm. internal diameter and 25.4 cm. in length were used in forced aeration experiments. These tubes were held upright with sterile air entering at the lower end.

All experiments, with one exception to be noted below, were carried out under controlled laboratory temperatures of $25° \pm 1.5°$ C.

GROWTH, SURVIVAL AND MORPHOLOGY UNDER LOW OXYGEN TENSIONS

Preliminary tests showed that the growth ratio between the two organisms in air approximated 2 to 1, with *Fusarium oxysporum* making the more rapid growth. Figure 1 shows how this ratio was modified to 4 to 1 when both organisms were placed in a large 6.5-liter desiccator from which all oxygen was removed. Sufficient oxygen was dissolved in the medium and in water used in maintaining high humidity to permit some growth. Four cultures of *F. eumartii* were dead when restored to air. Restoration growth was based on 2 cultures.

Further tests with initial oxygen tensions of 14.6 (two tests) and 51.1 mm./Hg. were conducted with the same desiccator. The lower tension yielded results similar to those given above even when the time of exposure was shortened to 6 days. The normal growth ratio of 2 to 1 was maintained under a time-tension relation of 3.25 days and tension of 51.1 mm./Hg. Growth was progressing when the cultures were removed to air. The presence of oxygen in chambers where growth had ceased could not be detected by the methods described above.

Subsequent tests with Hempel gas jars and a series of different oxygen tensions established simultaneously, showed that the growth rate of *Fusarium oxysporum* after removal to air was inversely proportional to the oxygen tension imposed at the beginning of the experiment. The explanation of this phenomenon as it occurs in invertebrates has been explained by Von Brand (25). He attributes it to an oxygen debt incurred by an organism during the period of stay in oxygen-poor surroundings. This results in an oxygen intake greater than usual after restoration to a normal oxygen tension. The surplus oxygen is used to eliminate non-oxidized or partially

oxidized products formed during the preceding period of partial asphyxiation.

Previous work by Goss[3] showed that *Fusarium eumartii* could not survive prolonged exposure to anaerobic conditions in pure culture. Accord-

GROWTH IN AIR
----- GROWTH UNDER LOW OXYGEN

F. OXYSPORUM

CONTROLS

F. EUMARTII

DIAMETER OF COLONIES (MM)

TIME (DAYS)
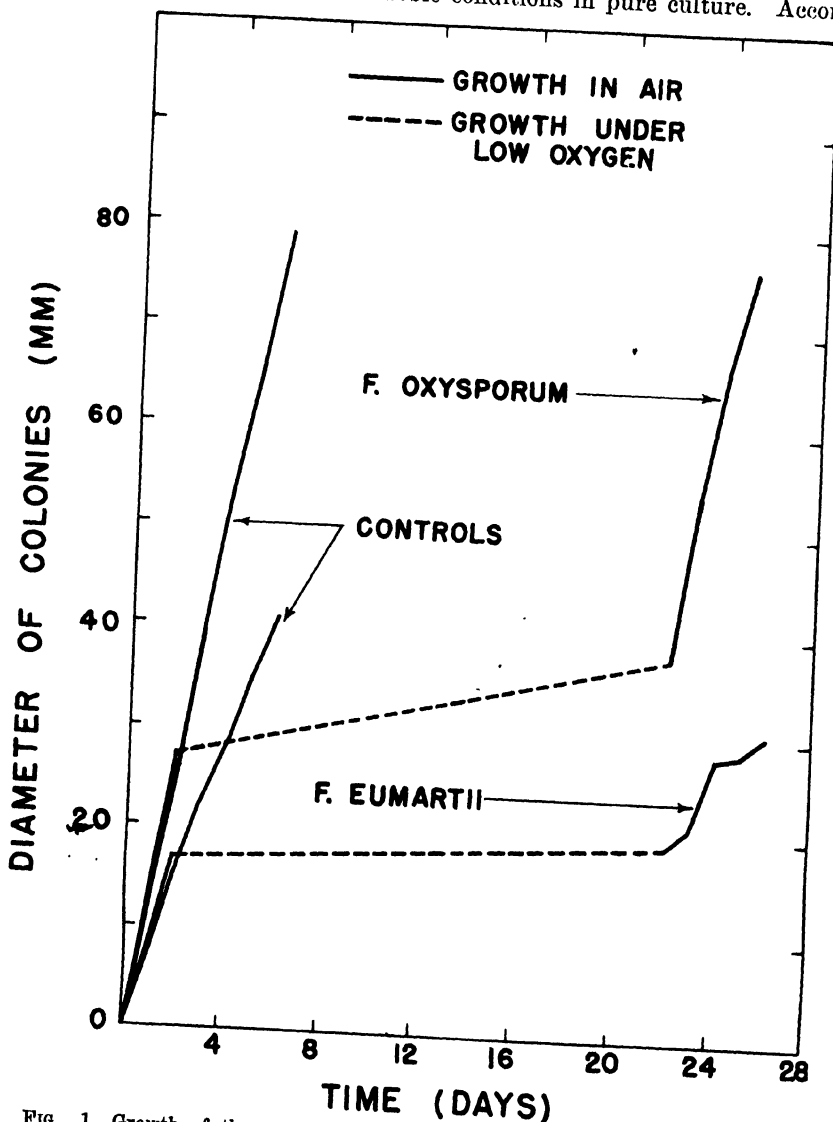
FIG. 1. Growth of the organisms under an initial oxygen tension of 0 mm./Hg. over a 20-day period compared with growth of control cultures.

ingly, 48-hour cultures were sealed in test tubes with paraffin and cork. Volatile substances were removed from the paraffin by holding it slightly above the melting point for one week. Control tubes were plugged with

[3] Goss, R. W. Unpublished work. Nebr. Agr. Exp. Sta.

cotton and covered with aluminum foil. All tubes were incubated at 25° C. for 13 weeks. Platings showed that all cultures of *F. oxysporum* were viable, whereas all cultures of *F. eumartii* were non-viable. The experiment was repeated for a period of 3 weeks and yielded identical results.

Morphological changes induced by hyphal tips of *Fusarium oxysporum* 293 growing under low oxygen tension are shown in figure 2, part A. Normal hyphae are provided for comparison. Both drawings are projection copies of photomicrographs. The first culture was placed under an initial oxygen tension of 73 mm./Hg. and held for 8 days. Similar growth was expressed under a range of oxygen tension-time relations, thus colonies started at initial tensions of 12, 31, and 73 mm./Hg. and held for 3, 4, and 8 days exhibited essentially the same morphologic response. Negative results at an extremely low oxygen tension were helpful in showing the nature of this response. Cultures placed in a chamber subjected to prolonged evacuation at an atmosphere pressure of 0.0003 mm./Hg. and retained there for 48 hours had a different morphology. Hyphal branching was repressed. The branches were short, averaging less than 10 μ in length.

Four types of laboratory media were used in these experiments in addition to potato-dextrose agar. The morphologic reaction was similar on all media tested.

Interesting observations were made of the recovery phenomenon in *Fusarium oxysporum* after removal of cultures to air. Cultures, seventy-two hours old, of *F. oxysporum* were subjected to an initial oxygen tension of 43.8 mm./Hg. and held for 3 days in a Hempel gas jar under a gradually decreasing tension. Nitrogen was added to make up the bulk of the atmosphere in the chamber. Microscopic examination showed that hyphal tips began to rupture and extrude their protoplasmic contents 2 hours after the colonies were restored to air. Rupturing of tips became more frequent between 2 and 3 hours after restoration. Normal hyphae began to elongate at the expense of their neighbors and growth along the entire margin was soon resumed. No rupturing of hyphal tips was observed after 15 hours. Morphologic changes of this character could not be induced in *F. eumartii*. All cultures had normal colony margins.

It was thought this morphologic response in *Fusarium oxysporum* 293 might be common to other species in the section Elegans. Accordingly, 5 additional isolates[4] falling into this section and 12 isolates[5] representing forms described by Snyder and Hansen (21, 22, 23) were tested. All results were negative. *F. oxysporum* 293 was the only isolate, of any tested, which gave a positive morphologic response to limiting oxygen tensions. The two additional Nebraska isolates of *F. oxysporum* have been cultured for more than 3 years, while *F. oxysporum* 293 has been cultured for more

[4] Two isolates of *Fusarium oxysporum cubense* from C. H. Meredith, one isolate of *F. lini* from J. J. Christensen, two additional Nebraska isolates of *F. oxysporum*.

[5] Forms of all species described, with the exception of *Fusarium nivale* (Fr.) Ces., received from W. C. Snyder. These included three forms each of *F. oxysporum* and *F. solani*.
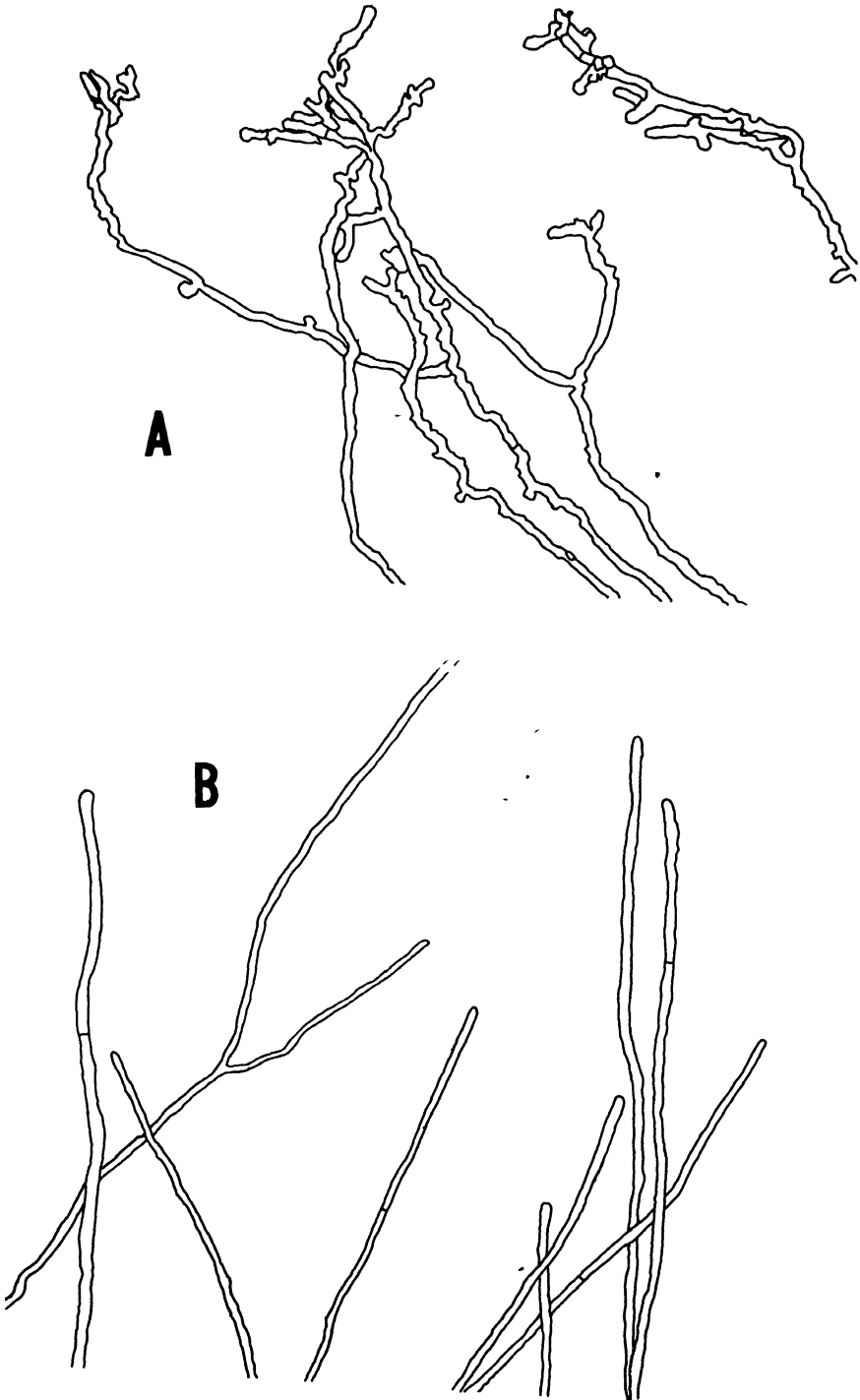
FIG. 2. Drawings of photomicrographs of colony margins of *F. oxysporum* 293 at 375 diameters. A. Morphologic reaction to an initial oxygen tension of 73 mm./Hg. B. Normal hyphae in free air.

than 10 years and the morphologic reaction peculiar to it has been observed for more than 6 years.

An experiment on growth-temperature relations at limiting oxygen tensions and temperatures of 10°, 15°, 20°, 25°, and 30° C. showed no shift in the optimum temperature for growth of *Fusarium oxysporum* on an artificial medium. Similarly, no measurable growth was recorded for *F. eumartii.*

The present data show that *Fusarium oxysporum* 293 ceases growth at an oxygen level well below 1 per cent and survives, while *F. eumartii* ceases growth at a higher oxygen level and dies under the anaerobic conditions that follow. While *F. oxysporum* 293 is utilizing oxygen, beginning at a level ranging from 3 to 10 per cent down to less than 1 per cent at the end of an experiment, definite morphological changes in the character of hyphal growth take place. These changes do not occur in *F. eumartii* when this organism is grown under the same conditions.

CARBON DIOXIDE RELATIONS

Preliminary experiments showed that these organisms were tolerant to a wide range of carbon dioxide concentrations. Accordingly, 8 desired concentrations ranging from 3.5 to 75.3 per cent were established. The general procedure has been described above.

The experimental unit was a Hempel gas jar containing 3 cultures of each organism. Four chambers were available making it possible to run 4 concentrations in each test. In order to maintain the desired carbon dioxide level and to measure daily growth, the concentrations were renewed at 24-hour intervals. Each test was repeated twice at nearly equal concentrations providing a total of 9 cultures.

Control cultures were held for each test as indicated. It must be stated that concentrations were randomized, *i.e.*, no particular sequence of carbon dioxide concentrations was employed in any one test.

Figure 3 represents the data from 6 successive experiments. The results show that both organisms made good, measurable growth over a wide range of carbon dioxide concentrations. Over the 4-day period the highest concentrations reduced growth of *Fusarium oxysporum* to less than one-fourth and in *F. eumartii* to about one-third of the normal amount, indicating that *F. oxysporum* is less tolerant .to high concentrations of carbon dioxide than *F. eumartii.*

Oxygen was suspected as a limiting factor at the highest carbon dioxide concentrations but calculation showed that the oxygen level was about 6.17 per cent when the carbon dioxide concentration was 75.3 per cent. Previous experiments showed that this is not a limiting oxygen value when the mixture is renewed daily in a chamber of this size. A generalized conclusion is that growth of neither organism can be much affected by the carbon dioxide in the soil atmosphere when the concentration is below 48 per cent. At this point the growth of *Fusarium oxysporum* was reduced by half while that of

F. eumartii was reduced by only one-third.   Figure 3 shows stimulation in the growth of *F. oxysporum* at about 3.5 per cent carbon dioxide.   Similar responses have been noted by Lundegårdh (10) for *F. culmorum* and *Gibberella saubinetii* in soil.
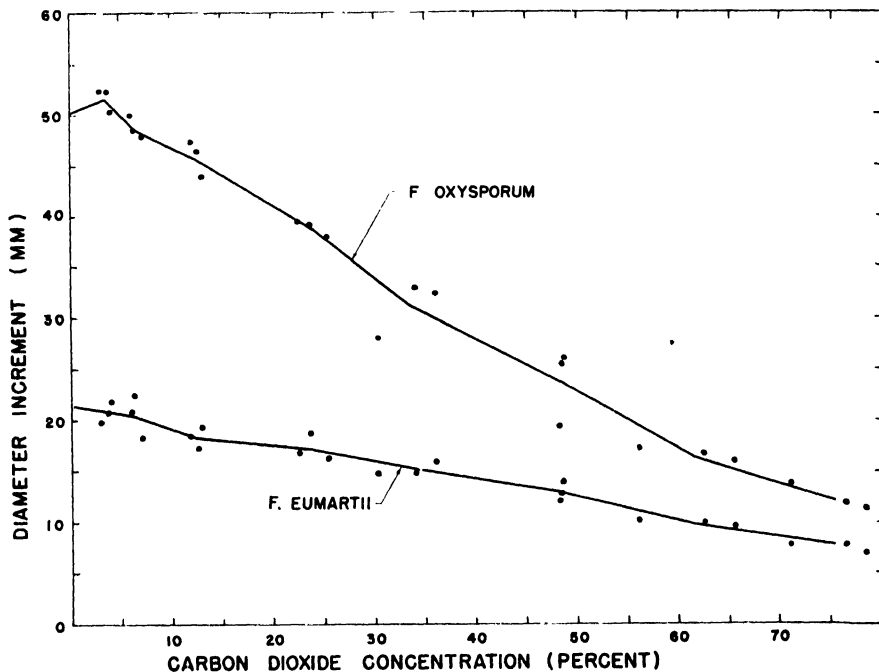
Fig. 3.   Comparative growth of the organisms over a 4-day period in pure culture at 25 different concentrations of carbon dioxide.

GROWTH IN SOIL

Preliminary experiments indicated that growth of the organism in soil is stimulated by air pockets.   Visible growth in the tubes was especially profuse in open areas between soil and tube.   This point is well illustrated by the results of an experiment conducted with sand of three particle sizes. The sand was washed in distilled water and passed through standardized screens to yield particles of the following size ranges: coarse, 1–0.5 mm.; medium, 0.5–0.25 mm.; and fine, 0.25–0.1 mm.   Moisture-holding capacity of each size series was determined by saturating 1 tube from each series, draining for 2 hours and reweighing.   The M.H.C. of coarse sand was 24.8 per cent; medium sand, 32.0 per cent; and fine sand, 34.3 per cent.

The tubes were saturated with a 5 per cent sterile sucrose solution and inoculated with a 0.5-ml. aqueous spore suspension.   To prevent drying of the sand and to limit passage of air into the tubes, parafilm paper was placed over the ends and held securely with rubber bands.   Three tubes were used for each organism and each sand particle size.   Growth between sand and tube was recorded after 10 to 20 days.   No significant differences could be

seen between growth of the two organisms but total growth in coarse sand during the second 10-day period was 15.3 cm.; in medium sand, 1.7 cm.; and in fine sand, 0.0 cm.

An experiment was made in which the independent variable was moisture content. A mixture of 60 per cent medium sand and 40 per cent coarse compost was adjusted to moisture levels in the tubes of 60, 80, and 100 per cent of M.H.C. Agar discs (1.5 cm.) from actively growing colonies were used for inoculations.

The results over a 4-week period were exceedingly variable. No differences in growth could be detected at different moisture levels but the growth ratio between the two organisms at all moisture levels approximated that

TABLE 1.—*Depth of isolations made from center of soil cylinder at conclusion of experiment with Fusarium oxysporum and F. eumartii growing in a sand-composted soil mixture*

Tube number	Organism		Percentage of M.H.C.	Viability of isolations from different depths		
				One-half visible growth	Visible growth	1 cm. beyond visible growth
No.	*Type*		*Per cent*	*Viability*	*Viability*	*Viability*
1	*F. oxysporum*		100	+	+	+
2	*F. oxysporum*		100	+	+	−
5	*F. eumartii*		100	+	+	−
6	*F. eumartii*		100	+	+	−
9	*F. oxysporum*		80	+	+	−
12	*F. oxysporum*		80	+	+	+
13	*F. eumartii*		80	+	+	+
14	*F. eumartii*		80	+	+	+
17	*F. oxysporum*		60	+	−	−
18	*F. oxysporum*		60	+	+	+
21	*F. eumartii*		60	+	+	+
22	*F. eumartii*		60	+	+	+

obtained in pure culture. The average weekly increment of *Fusarium oxysporum* was approximately 20 mm. while that of *F. eumartii* was about 12 mm. Table 1 shows the results of isolations made on Burkholder's medium. Greatest penetration of the soil cortex by both organisms was made at moisture levels of 60 and 80 per cent of M.H.C. Bacterial contamination was present in tubes held at 100 per cent of M.H.C. A similar experiment was conducted using large tubes and forced aeration. Moisture levels in uncomposted top soil were 40, 60, and 100 per cent M.H.C., which was determined as 53 per cent of oven-dry weight. The tubes were plugged with cotton at both ends and inoculated with a 1-ml. spore suspension and a 20-mm. agar disc inverted on top of the soil columns and covered with moist sterile sand. The aeration train for each tube consisted of a concentrated NaOH trap and a water-bottle used for regulating the rate of air flow with a screw clamp. This arrangement was later used to determine CO_2 evolution by replacing water with alkali.

Isolations were made from 6 levels in each tube after 24 days onto acidulated and neutral Burkholder's medium (Table 2). Again, the best penetration was made at medium and low moisture levels. Bacterial contamination was present again in tubes held at 100 per cent of M.H.C. No antagonism between the fungi and bacterial contaminants could be detected in pure culture.

Activity of the organisms in composted soil with a M.H.C. of 37.5 per cent was studied by determining carbon dioxide evolution over an 8-day period. Moisture content of the soil was adjusted to 48 per cent of M.H.C. The tubes were sterilized for 1 hour at 15 lb. pressure on three alternate days over a 6-day period. Six of the 8 tubes were inoculated with a 1-ml. spore suspension and the remaining 2 were held as controls. After 7 days, 5 and 15 ml. of sterile water were added to the first two tubes of each series,

TABLE 2.—*Depth of isolations made from center of soil cylinder at conclusion of experiment with Fusarium oxysporum and F. eumartii growing in uncomposted top soil under forced aeration*

Tube number	Organism	Percentage of M.H.C.	Viability of isolations					
			Depth (cm.)					
			2	4	6	8	12	15
No.	*Type*	*Per cent*	*Viability*					
1	F. oxysporum	100	+	+	–	–	–	–
2	F. eumartii	100	+	–	–	–	–	–
3	Control	100	–	–	–	–	–	–
4	F. oxysporum	60	+	+	+	+	+	+
5	F. eumartii	60	+·	+	+	–	–	–
6	Control	60	–	–	–	–	–	–
7	F. oxysporum	40	+	+	+	+	+	–
8	Control	40	–	–	–	–	–	–

respectively, to determine if this would result in an increase in activity over the eighth day. The remaining tubes were weighed to determine water loss.

The initial rise (Fig. 4) was probably due to the stimulus of inoculum and CO_2 trapped in the system at the start of the experiment. Weighing of unwatered tubes showed a water loss ranging from 2 to 5 gm. This represents an average loss of about 13 per cent. This loss, coupled with the low initial moisture level, accounts for the low level of activity over the course of the experiment. The increase in CO_2 evolution over the last day again can be attributed to two factors: CO_2 trapped in the system and increased activity of the organisms.

Growth of the organisms between a glass surface and sand of different particle size showed that it is possible to control the rate of growth with size of sand particle. The results do not show whether this is primarily due to air content or soil space. High nutrient level was eliminated as a factor because greatest growth occurred in the presence of lowest nutrient supply. Tolerance of the organisms to lowered oxygen tensions in pure

culture coupled with the results discussed above, suggest that the size of soil spaces may be an important factor determining growth in soil.   Meredith (11) found that *Fusarium oxysporum cubense* made an average daily growth on the surface of greenhouse compost in long glass tubes of 0.47 cm. per day, whereas the daily average on clay was only 0.3 cm.   Here the effects of
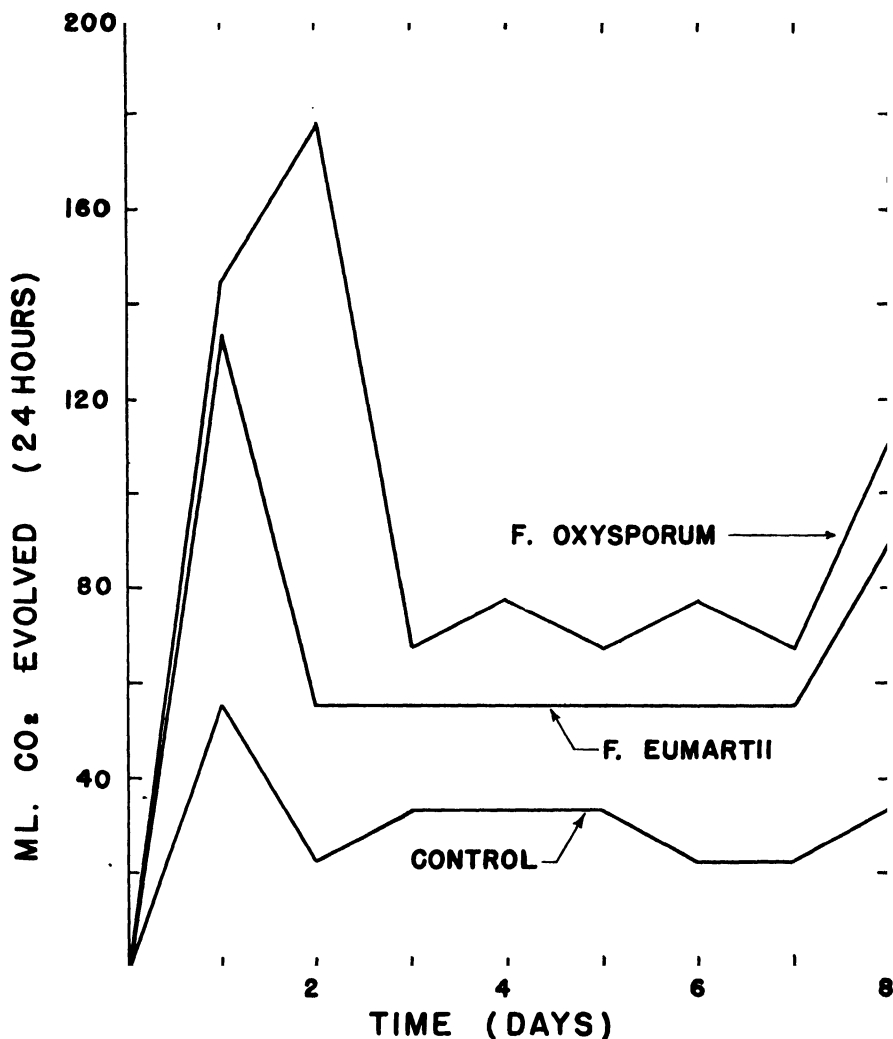
FIG. 4.   Carbon dioxide evolution by the organisms in greenhouse compost at approximately 48 per cent of M.H.C. (M.H.C. 37.5 per cent of dry weight).

air content must have been negligible since the tubes were only half filled with soil.   Nutrient level and soil space may have determined primarily the rates of growth.

Greater penetration of both unaerated and aerated soil at lower moisture levels by *Fusarium oxysporum* and *F. eumartii* is more difficult to explain. Higher moisture levels may have limited growth indirectly by cutting down

soil space as well as air content. This could have occurred with swelling of the organic colloids and their cementing together with bacterial gum. Meredith (11) found that sandy soils permitted greater penetration by *F. oxysporum cubense* than loamy soils. Both soil space and air content may have been important in bringing about this difference.

The organisms used in this study are capable of independent growth in the soil similar to that demonstrated by Blair (1) for *Rhizoctonia solani* Kühn. Growth proceeds primarily through spaces in the soil and along solid surfaces.

<div align="center">DISCUSSION</div>

This work has emphasized the tolerance of both organisms to oxygen and carbon dioxide. Survival under anaerobic conditions and greater relative growth under low oxygen tensions indicate that *Fusarium oxysporum* is a more versatile organism than *F. eumartii*.

It is impossible to draw conclusions from the present data relative to allied species or forms in the sections Elegans and Martiella. It was seen, for example, that a positive morphologic response to lowered oxygen tensions by *Fusarium oxysporum* 293 was absent in 2 other isolates of *F. oxysporum* and in 17 other species and forms of the genus tested. Different isolates of the same species might also differ from one another in their ability to grow under low oxygen tensions.

Nowhere have differences between species and forms of the genus been expressed as in the work of Reinking and Manns (16, 17) and Reinking (14, 15). The first study (16) located in Honduras and Guatemala included some 15 soil types comprised mostly of sandy-clay and loam mixtures. It was reported that 24 different species, varieties, and morphologic forms of the genus *Fusarium* were isolated from surface soil in the areas sampled. The greatest number of types were from the section Elegans and 3 of these types were found in nearly all surface soils. It is interesting that *F. solani* var. *eumartii* was found to occur in only a few soil types, but was found commonly in isolations from all depths examined. Indeed, they classified this organism as a soil inhabitant on the basis of isolations from depths of the soil below a half inch. There is little agreement between their findings and those presented in this paper. There is, however, agreement between relative frequency of forms of *F. oxysporum* and the more widespread occurrence of oxysporum wilts as compared with the more sporadic distribution of Martiella species and their corresponding root rots. It is generally recognized that oxysporum wilt of potato is more widespread than eumartii wilt.

Reinking and Manns (14, 15) further found that the majority of *Fusarium* spp. obtained were isolated in greater relative numbers from light acid soils. Concerning the distribution of Panama disease, Wardlaw (26) states in part, "The collective experience, practical and scientific, in Central America is that hydrogen ion concentration is the master factor determining

the severity of Panama disease, followed by texture." Recently Wardlaw (27) reported results of an interesting experiment carried out in Honduras. Land which had been removed from cultivation, because of the Panama disease (*Fusarium oxysporum cubense*), was divided into sections and flooded for periods ranging from 6 to 18 months. The fungus presumably died of oxygen starvation, since control of the disease was secured for as long as 6 years after treatment although isolated plants were found to be infected after 10 to 18 months. While the present results are at variance with this report, it must be remembered that we are dealing with related organisms which were tested in two different substrates. The author's own data warrant the speculation that antibiosis may have been important in eliminating *F. oxysporum cubense* from this flooded soil and in line with the phenomenon of recovery above, that frequent short periods of flooding might be more effective in promoting antagonism to the fungus than one prolonged period. Wardlaw further states, "It is considered improbable that the method of flood-following will ever be successful in acid soils (in which plants succumb readily and in which the fungus may spread with great rapidity), but the system is being extended to other areas considered to be suitable." The present results suggest that soil space as controlled by texture may be a more important factor in the distribution of these organisms than soil air content. More work is needed to separate the effects of texture and air content upon fungus growth in soil.

We cannot account for differences in ecology of the organisms on the basis of their reaction to oxygen since both can grow at extremely low tensions, but these results indicate that *Fusarium oxysporum* is better equipped for survival as a saprophyte than *F. eumartii* and that the latter organism might tend to die out more readily in a water-logged soil.

SUMMARY

1. The effects of different oxygen and carbon dioxide concentrations upon growth and morphology of *Fusarium oxysporum* and *F. eumartii* were studied in pure culture. Growth in sand and various mixtures of sand and compost was studied in relation to moisture content, texture, and forced aeration.

2. *Fusarium oxysporum* had greater tolerance to lowered oxygen tensions than *F. eumartii*.

3. Sealed cultures of *Fusarium oxysporum* were viable after 13 weeks, whereas those of *F. eumartii* were non-viable after 21 days.

4. Both organisms had considerable tolerance to high carbon dioxide concentrations.

5. *Fusarium oxysporum* 293 exhibited a definite morphologic reaction to lowered oxygen tensions in pure culture.

6. This morphologic reaction did not occur in any of 19 additional isolates of the genus *Fusarium* including 2 isolates of *F. oxysporum*.

7. In an unaerated sand-composted soil mixture nearly equal penetration was obtained at all moisture levels, with the growth ratio approximating that obtained in pure culture.

8. In both unaerated and aerated soil, greater penetration was obtained at moisture levels below saturation.

9. Under forced aeration in composted soil the organisms were active at moisture levels below 48 per cent of moisture-holding capacity.

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YELLOWS AND NECROTIC RING SPOT OF SOUR CHERRIES IN ONTARIO[1]—DISTRIBUTION AND SPREAD

R. S. Willison, G. H. Berkeley and
G. C. Chamberlain

Under one name or another, yellows of sour cherries has been known in Ontario and elsewhere for many years. The disease had become widely distributed in cherry growing districts before 1939, when its infectious nature was demonstrated by Keitt and Clayton (6) in Wisconsin. Moreover, the absence of definite symptoms in late summer makes it difficult to propagate yellows-free nursery stock, unless great care is exercised in the selection of parent trees. There is, therefore, good reason to believe that most of the present plantings of sour cherries have been more or less contaminated with yellows from the start. On the other hand, the annual recurrence of symptoms in late June (7), though varying greatly in intensity from season to season, makes it possible to follow with comparative ease the progress of the disease in an orchard over a period of years.

In Ontario, the first known cases of necrotic ring spot were observed on Montmorency cherry in the spring of 1939 (3), and the virus nature of the disease was demonstrated soon afterwards. At about the same time, necrotic ring spot, or a very similar disease, was discovered independently in various parts of North America (4, 5, 10) on sour cherry or other hosts. The symptoms of the disease have been considered in detail elsewhere (1, 3, 4, 5) and need not be discussed here, except to mention the apparent recovery of affected trees soon after infection. Furthermore, indexing experiments (1) have shown that many apparently healthy sour cherry trees carry necrotic ring spot in latent form and, on the other hand, that a few do not. It would thus appear that this disease, even more than yellows, is frequently and unwittingly propagated in the nursery and set out in the orchard. Because of the tendency to recovery and the consequent irregular occurrence of diagnostic symptoms after the first year of infection, there is considerable uncertainty concerning both the actual incidence of necrotic ring spot in a given orchard and the rates and conditions of spread, facts which could be determined with any degree of accuracy only by the well-nigh impossible task of indexing every apparently healthy cherry tree in the orchards under consideration. The present paper is an attempt to come to some conclusion on these points by means of an analysis of data obtained from orchard surveys.

MATERIAL

Since 1941, a number of Montmorency cherry orchards, one for yellows alone, two for necrotic ring spot alone, and three for both diseases, have

been under observation for three or more successive years.   Each orchard
was charted to show the known distribution of each disease at the time of
the first survey and the location of the new cases appearing in each succeed-
ing year.   In addition, the distribution of yellows was mapped in five more
orchards for the first time in 1945.

INCIDENCE

The incidence of each disease in the several orchards in the first survey
and in 1945, as well as the annual increases were calculated as percentages
of the number of trees per orchard (Tables 1 and 2).   Since, for reasons
already mentioned, the significance of the necrotic ring spot data may be
open to question, it was considered advisable to submit alternative inter-

TABLE 1.—*Percentages of Montmorency cherry trees in various orchards infected
with yellows, at the first survey, and becoming infected each year thereafter*

Orchard No.	Age in 1945	No. trees	Initial incidence	New infections				Mean increase per annum	Incidence in 1945
				1942	1943	1944	1945		
1	15	432	22.0[a]	3.2	11.8	9.7	20.8	11.4 ± 3.6	67.5
2	8	288	3.5	—[b]	—	1.4	6.6	4.0 ± 2.6	11.5
3	12	72	2.8	5.5	4.2	2.8	12.5	6.3 ± 2.2	27.8
4	20	138	9.4	2.2	4.7	2.3	2.4	2.9 ± 0.6	21.0
Mean (orchards 1–4)				9.4 ± 4.5	3.6 ± 1.0	6.9 ± 2.5	4.1 ± 1.9	10.6 ± 4.0	6.4 ± 1.5[c]	31.9 ± 12.3
5	7	66	—	—	—	—	—	—	45.5
6	10	180	—	—	—	—	—	—	27.7
7	3	388	—	—	—	—	—	—	18.8
8	3	224	—	—	—	—	—	—	26.8
9	18	84	—	—	—	—	—	—	100.0
Mean incidence in 1945 (orchards 1–9)									38.5 ± 9.5

[a] In tables 1 and 2 initial incidence is for the year immediately preceding the first
record of new infections, *e.g.*, in orchard 2, the incidence of yellows was 3.5 in 1943.
[b] —, No records taken.
[c] Mean of all readings of new infections.

pretations, each serving as a check on the other.   The first interpretation,
based on the assumption that new cases arose in the year in which symptoms
were first detected or suspected, gives what might be termed "apparent an-
nual spread."   If, however, the severity of the observed symptoms is taken
into account, as befits the known characteristics of necrotic ring spot, the
"probable annual spread" can be computed.   For this purpose, the few
trees in which infection was only suspected were regarded as "healthy,"
while those exhibiting only mild symptoms were classified as having been
affected before the surveys began and those with severe or moderately
severe symptoms as representing the new infections.   Since neither inter-
pretation solves the problem of the health status of the trees in which no
symptoms were observed, only the "minimum incidence" of necrotic ring
spot in each orchard can be known.   The assigned values of minimum in-

TABLE 2.—*Percentages of Montmorency cherry trees in various orchards, known to be infected with necrotic ring spot at the first survey, and becoming infected each year thereafter*

Orchard No.	Apparent incidence and spread[a]						
	Initial incidence (minimum)	New infections				Mean increase per annum	Incidence in 1945
		1942	1943	1944	1945		
1	42.8	—	—	11.3	5.8	8.6 ± 2.8	60.0c
2	42.0	—	—	7.0	13.5	10.3 ± 3.2	62.5
3	4.2	20.8	25.0	22.2	0.0	17.0 ± 5.7	72.2
5	27.3	—	18.2	9.1	3.0	10.1 ± 4.4	57.6
6	17.8	—	—	12.2	9.4	10.8 ± 1.4	39.4
Mean	26.8 ± 7.3	20.8	21.6 ± 3.4	12.4 ± 2.8	6.3 ± 2.4	12.1 ± 2.1	58.3c ± 5.4

Orchard No.	Probable incidence and spread[b]							
	Initial incidence (minimum)	New infections					Mean increase per annum	Incidence in 1945
		1941	1942	1943	1944	1945		
1	34.5	—	—	14.6	5.8	1.8	7.4 ± 3.8	56.7c
2	24.6	—	—	19.5	5.2	6.6	10.4 ± 4.6	55.9
3	29.3	5.5	18.0	13.9	5.5	0.0	8.6 ± 3.2	72.2
5	16.7	—	24.2	6.1	6.1	3.0	9.9 ± 4.8	56.1
6	26.7	—	—	0.0	3.3	6.1	3.1 ± 1.8	36.1
Mean	26.4 ± 2.9	5.5	21.1 ± 3.1	10.8 ± 3.4	5.2 ± .5	3.5 ± 1.3	8.1 ± 1.6	55.4c ± 5.7

[a] Infections date from year in which any symptoms were first observed.
[b] Infections date from year in which severe symptoms occurred; trees with mild symptoms classified under initial incidence.
[c] Calculations of apparent incidence and spread include suspected cases; those of probable incidence and spread do not.

cidence for any year would of course vary according to the interpretation of the data. On the other hand, comparison of the two points of view is of value in considering the rate and manner of spread.

Since both diseases are infectious, it was thought that the amount of disease present in an orchard at any time would have an important bearing on rate of spread. Accordingly the incidence of each disease in indi-

TABLE 3.—*Coefficients of correlation between the percentage of diseased trees in an orchard in any one year and the percentage of new infections appearing the next year*

Disease	A[a]		B[b]	
Cherry yellows	0.729	(1 per cent)c	0.815	(< 1 per cent)
Necrotic ring spot				
(apparent spread)	−0.656	(2 per cent)	−0.331	(25–30 per cent)
(probable spread)	−0.503	(3 per cent)	−0.276	(25–30 per cent)

[a] Correlations using percentage increases calculated in terms of the number of trees in the orchards (See tables 1 and 2).
[b] Correlations using percentage increases calculated in terms of the number of "healthy" trees available for infections. The percentages of previously diseased trees were the same for both columns.
[c] Levels of significance.

vidual orchards in each year was correlated with the corresponding amount of spread in the next year (Table 3). The first column of coefficients was calculated from the information given in tables 1 and 2. However, the fact that the percentage of trees to which the disease can spread in a given orchard decreases automatically as the incidence rises, tends to give those correlations a negative bias. To correct this tendency, incidence was also correlated, as shown in the last column of table 3, with percentage increases calculated in terms of the number of trees remaining "healthy" the previous year (Table 4).

With yellows, there was a wide variation both in incidence from orchard to orchard and in the rates of spread in different orchards and seasons (Tables 1 and 4). It might be expected that older orchards would be more severely attacked than younger ones, but the 1945 incidence of yellows was not significantly correlated with age of orchard. Also, the rates at which the disease increased were independent of the age factor. Similar results were obtained by Keitt and Clayton (7), who reported an average increase of 3.5 per cent per annum in five orchards during the year 1936 to 1940. It is scarcely necessary to point out that the current percentage of affected trees in an orchard depends not only on the length of time to which the trees have been exposed to infection, but also on the number and location of diseased trees set out with the orchard, on the presence or absence of other sources of inoculum, on the nature and prevalence of the disseminating agent or agents, and on other factors which may influence the annual rate of spread. The highly significant correlation between the amount of spread and the amount of disease previously present (Table 3) indicates that the probability of the spread of yellows from tree to tree increases with the number of sources of infection and confirms by inference the view that trees which do not exhibit symptoms may be presumed yellows-free. On the other hand, since the average annual rates of increase per orchard were not proportional to the initial observed incidence (Tables 1 and 4), the rate of spread of yellows in an individual orchard was evidently modified by some other factors of local significance.

The successive patterns of distribution of yellows-infected trees in each block (Fig. 1) provide some evidence that the relative position of sources of infection in an orchard can also play an important part in the later history of the disease in that orchard. For example, the diseased trees, at the time of the first survey in 1941, were massed along the north side and towards the west end of orchard 1 (Fig. 1). In orchard 2, a younger block adjoining the south side of orchard 1, the first diseased trees were mostly scattered singly and at random. Here the average increase of yellows per annum was much less than in orchard 1 (Table 1). The later surveys showed that new infections in both blocks tended to arise with greater frequency to the east and south of potential sources than to the west and north. There also appeared to be some easterly and southerly drift of infection from the older orchard to the adjacent half of the

TABLE 4.—Percentages of previously "healthy" trees becoming diseased in the year indicated

Orchard No.	Cherry yellows										Necrotic ring spot					
	Increase in				Av. increase per annum	Apparent increase in[a]				Av. increase per annum	Probable increase in[b]					Av. increase per annum
	1942	1943	1944	1945		1942	1943	1944	1945		1941	1942	1943	1944	1945	
1	4.2	15.8	15.4	39.1	18.6 ±7.4	—	—	19.8	12.6	16.2 ±3.6	—	—	22.2	11.4	4.1	12.6 ±5.3
2	—	—	1.5	6.9	4.2 ±2.7	—	—	12.0	26.5	19.3 ±7.3	—	—	25.8	9.3	13.0	16.0 ±5.0
3	5.7	4.5	3.2	14.8	7.1 ±2.6	21.8	33.3	44.4	0.0	24.9 ±9.5	7.8	26.6	29.4	16.7	0.0	16.1 ±5.6
4	2.4	4.9	2.8	2.9	3.3 ±.6	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	.6	—	25.0	16.6	6.7	16.1 ±5.3	—	29.1	10.3	11.4	6.5	14.3 ±5.0
6	—	—	—	—	—	—	—	14.9	13.5	14.2 ±.7	—	—	0.0	4.5	8.7	4.4 ±2.5
Av.	4.1 ±1.0	8.4 ±3.7	5.7 ±3.2	15.9 ±8.1	8.9 ±2.7	21.8	29.2 ±4.2	21.5 ±5.9	11.9 ±4.2	19.0 ±3.3	7.8	27.8 ±1.3	17.5 ±5.4	10.7 ±2.0	6.5 ±2.2	13.2 ±2.3

a New infections date from year in which any symptoms were first observed.
b New infections date from year in which severe symptoms occurred; trees with mild symptoms classified under initial incidence. (See table 2.)

younger one. In orchard 3, in which a relatively rapid increase occurred, the first new infections appeared to the north of one of the original sources (Fig. 1). These possibly came from an adjoining sweet cherry orchard
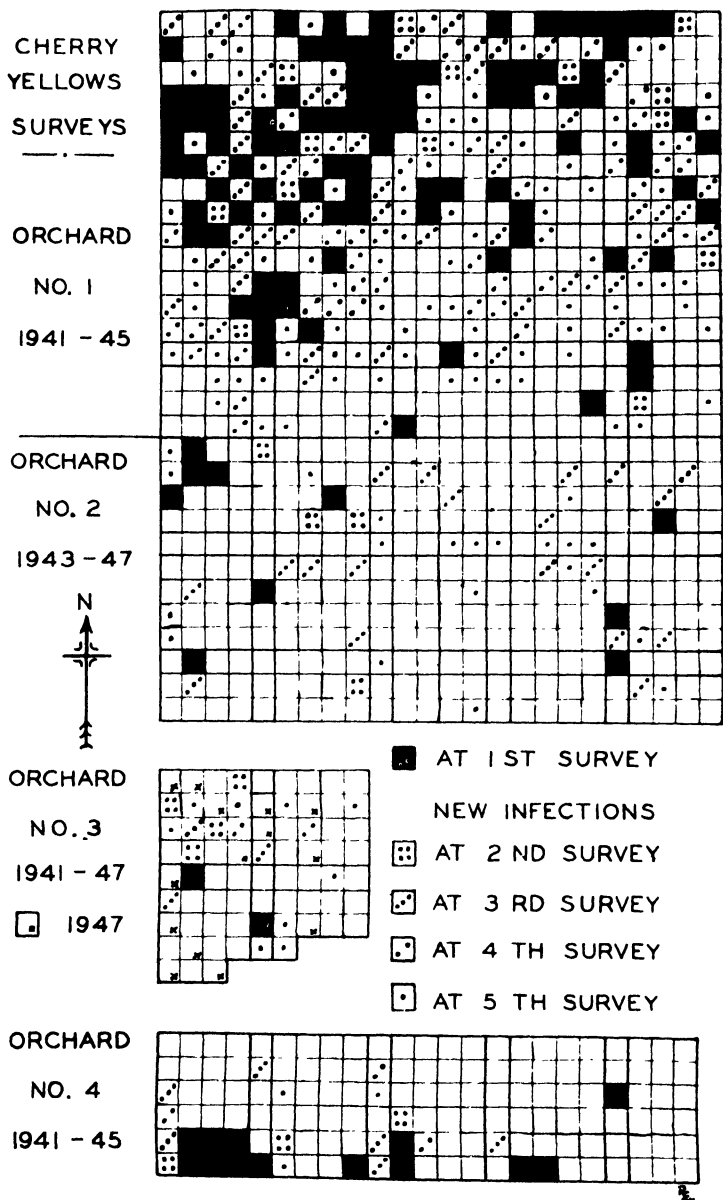
FIG. 1. Diagrammatic maps of four Montmorency cherry orchards showing the distribution of cherry yellows at the time of the first survey and the location of new infections observed during each subsequent survey.

to the west, in which there were scattered a few sour cherries. A group of diseased trees was thus established in the northwest corner, so that con-

ditions here became similar to those in orchard 1. The eastward and south-
ward drift of infection became apparent in the third and subsequent sur-
veys. Orchard 4 is of particular interest, first, because it had the lowest
rate of spread, in spite of its being the oldest planting and having the sec-
ond highest initial incidence, and secondly, because the situation here was
almost the reverse of that in orchard 1. The original sources of infection
in this orchard were evidently distributed along its southern edge and the
spread of infection into the interior of the orchard was comparatively slow
(Fig. 1). If it is taken into consideration that, in this region, the pre-
vailing winds are from the west to southwest and that the strongest winds
most frequently blow from the northwest, it would appear that the location
of infection sources with respect to the prevailing winds, as well as the
number and degree of concentration of sources, had some influence on the
rate at which cherry yellows spread through the several orchards under
survey. That is to say, the disease tended to increase more rapidly where
the sources of infection happened to be located on what was usually the
windward side of the orchard than where they were located on the lee-
ward side.

The foregoing observations suggest that an insect vector whose move-
ments can be influenced by air currents is likely to be of major significance
in the dissemination of cherry yellows in an established orchard. If so,
local and seasonal fluctuations in vector populations could exert further in-
fluence on rates of spread of yellows in different years and localities.

That the intensity and prevalence of symptoms of cherry yellows de-
pends mainly upon the temperatures prevailing during leaf development
in sour cherry trees was demonstrated by Keitt and Moore (8) in green-
house experiments. Mills (9) has also shown that variation from year to
year in the degree of symptom expression of yellows in the orchard can be
correlated with the temperatures obtaining during the 30-day period fol-
lowing petal-fall. Consequently, seasonal differences in the annual increase
of yellows can be to some extent attributed to the fact that in some years
symptom expression may be partially suppressed. In 1944, for example,
some new cases could easily have been overlooked and so might not appear
in the records until the following year. On the other hand, since symp-
toms were both prominent and prevalent in 1941 and again in 1945 and
only slightly less so in 1942 and 1943, it is reasonable to conclude that the
assessment of the yellows status of the various orchards is substantially ac-
curate for the complete survey period, and for most of the individual years
concerned.

Necrotic ring spot incidence appears to differ from that of yellows in
several respects. In the first place, both initial and current percentages
of trees affected with necrotic ring spot were higher in most individual or-
chards as well as in average and, besides, varied less widely from orchard
to orchard (Table 2). This would suggest either that more trees were af-
fected with necrotic ring spot than with yellows at planting time or that

the former disease spread with greater rapidity. At the present stage of investigation there is no way of ascertaining the full effects of the first assumption, except by deduction, though it has some foundation as shown
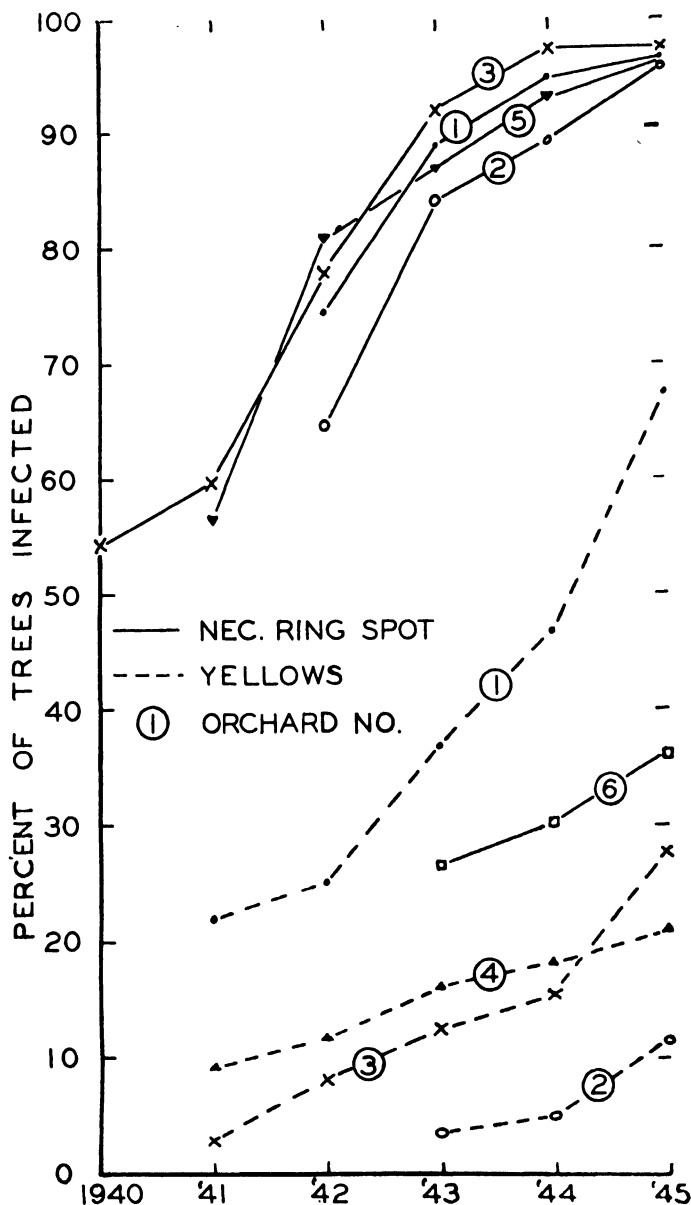
FIG. 2. Percentages of cherry trees, in various orchards, infected with yellows and with necrotic ring spot in successive years. The curves for necrotic ring spot are based on figures for probable spread and adjusted to allow for the occurrence of masking.

by comparison of the number of groups of diseased trees (Table 6). The possibility that necrotic ring spot spreads more rapidly than yellows ap-

TABLE 5.—*Adjusted incidence of necrotic ring spot and the corresponding percentages of previously "healthy" trees becoming diseased*

Orchard No.	Initial incidence	Adjust-ment	Adjusted incidence						Probable increase (Percentage of "Healthy")					Average
			1940	1941	1942	1943	1944	1945	1941	1942	1943	1944	1945	
1	34.5	+40	—	—	74.5	89.1	94.9	96.7	—	—	57.2	53.2	36.4	48.9 ± 6.4
2	24.6	+40	—	—	64.6	84.1	89.3	95.9	—	—	54.9	32.6	61.3	49.6 ± 8.7
3 (a)	29.2	+25	54.2	59.7	77.9	91.8	97.2	97.2	12.1	44.8	62.4	66.6	0.0	37.2 ± 13.4
(b)		+27.8	57.0	62.5	80.7	94.6	100.0	100.0	12.9	48.2	71.5	100.0	—	58.2 ± 18.4
5	16.7	+40	—	56.7	80.9	87.0	93.1	96.1	—	55.2	30.8	44.4	40.0	42.6 ± 5.1
6	26.6	+0	—	—	26.6	26.6	29.9	36.0	—	—	0.0	4.5	8.7	4.4 ± 2.5

Coefficient of correlation between adjusted incidence and subsequent spread, +0.528 (3–4 per cent), using 3 (a), or +0.683 (< 1 per cent) if orchard 3 were completely infected in 1944 (see 3 (b)). There were no new infections in orchard 3 in 1947.

pears to be confirmed by the data given in table 4, since, on the average, only 8.9 per cent of the "healthy" trees became infected with yellows each year and between 13.2 and 19.0 per cent with necrotic ring spot. These comparisons, however, may not be altogether valid for reasons which are developed later. In the second place, the rates of spread of necrotic ring spot were correlated negatively, instead of positively, with the prevalence of infective sources (Table 3). This inversion of correlation, which is contrary to what would normally be expected of an infectious disease with a relatively high rate of spread, and the contingent progressive falling off of the annual increase in most of the orchards (Tables 2 and 4) are only understandable in view of the masking of necrotic ring spot and of the consequent probability that a considerable proportion of the "healthy" trees were infected before the survey began. Furthermore, if the probable incidence per annum is plotted for individual orchards (Fig. 2), the resultant graphs, except that for orchard 6, take the shape of the upper part of a characteristic sigmoid curve. This type of curve also indicates the probability that necrotic ring spot infection in four of the orchards was approaching the saturation point by 1945.[2] In accordance with this hypothesis, the positions of the curves (Fig. 2) have been adjusted, where adjustment appears to be warranted, to bring the 1945 incidence above 95 per cent (Table 5). The corresponding curves for yellows incidence, and for necrotic ring spot incidence in orchard 6, (Fig. 2) are typical of the lower or middle parts of sigmoid curves, in agreement with the comparatively low or medium degrees of infection observed. The probable spread associated with the adjusted incidence of necrotic ring spot (Table 5) is several times that associated with the observed minimum incidence (Table 4) and shows a significantly positive correlation with the adjusted incidence of the previous year (Table 5). Thus it would appear that the rates of spread, whether as percentages of the total number of trees or as percentages of previously "healthy" trees, is not the same for different levels of infection. It is therefore doubtful if any mean rate of annual increase can be established for either disease. Certainly comparisons should not be made without considering incidence levels. The possible effects of prevailing winds on the spread of necrotic ring spot were obscured by the wide distribution throughout each orchard of groups of trees known to be diseased at the time of the first survey.

DISTRIBUTION OF DISEASED TREES IN ORCHARDS

In their studies of the distribution of psorosis in citrus orchards, Bitancourt and Fawcett (2) devised means of analysing maps compiled in single surveys. By this means they deduced, as they were later able to demonstrate, that psorosis may be transmitted in the orchard by natural root

[2] In support of the hypothesis that 4 of the 5 orchards were close to the saturation point for necrotic ring spot, it may be noted that in 1947 the incidence of new infections ranged from 0.0 to 0.5 per cent (of totals) in orchards 1, 2, 3, and 5, but was 10.8 per cent in orchard 6.

grafts. In their method of analysis, the average number of diseased trees at certain distances around each diseased tree was compared with the average number of diseased trees at the same distances around healthy trees. This was done in order to disclose possible patterns of spread from sources introduced unknowingly into the orchard at planting. The distances involved were 1 L, 1.4 L, 2 L, and 2.8 L, where L is the planting distance. In the present studies 2.2 L and 3 L+ were also included. The relative positions of trees at these distances from a central tree are shown in figure 3. However, the method of analysis was modified to take advantage of data obtained in a series of surveys. From information of this type it was possible to calculate for each disease not only the percentage of diseased trees at each distance from the nearest source, but also the percentage of "healthy" trees both available for later infection and becoming infected at these respective distances. It should be understood
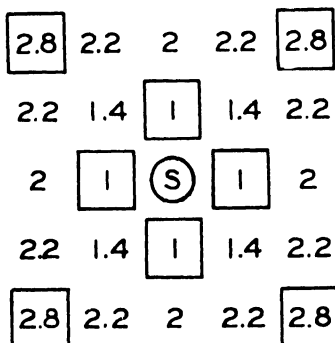
FIG. 3. Location of trees at various distances 1 L to 2.8 L from a central tree, S, where L = planting distance. All trees outside this pattern are classified as 3 L+.

that, in presenting the results of this analysis it is intended to show the probability of occurrence of spread over various minimum distances and not to imply that spread invariably followed the patterns indicated.

The distributions of diseased and "healthy" trees, with respect to the nearest diseased tree, in the various orchards at the time of the first survey are presented in table 6. The relatively high percentages of diseased trees adjacent to each other are interpreted as evidence of short distance spread taking place before the surveys began and resulting in groupings of affected trees, particularly where incidence was low, for example, the groupings of initial yellows infections in orchard 4. It should also be observed that the opportunities for short distance spread to "healthy" trees become more and more prevalent as more trees become infected (Table 6). High percentages of diseased trees at 3 L+ may arise either from spread or from the distribution of trees infected when set out, for example, yellows in orchards 2 and 3.

The probable distribution of spread can be determined with greater accuracy by considering the relative frequency with which new cases occur at various distances from the nearest source of infection in the second

TABLE 6.—*Percentages of diseased and of healthy trees to be found at different distances from the nearest diseased tree, and the number of groups of diseased trees at the time of the first survey*

Orchard No.	Initial incidence	No. of groups	Distribution											
			of diseased trees at						of "healthy" trees at					
			1 L[a]	1.4 L	2 L	2.2 L	2.8 L	3 L+	1 L	1.4 L	2 L	2.2 L	2.8 L	3 L+
Cherry Yellows														
1	22.0	20	75.8	14.7	6.3	1.1	0.0	2.1	43.9	17.8	12.7	11.6	3.3	10.7
2	3.5	6	30.0	10.0	20.0	0.0	0.0	40.0	11.2	9.7	9.3	16.2	5.4	48.2
3	2.8	2	0.0	0.0	0.0	0.0	0.0	100.0	11.4	11.4	8.6	10.0	4.3	54.3
4	9.4	4	84.6	0.0	7.7	0.0	0.0	7.7	17.6	9.6	12.8	14.4	6.4	39.2
5	45.5	6	80.0	20.0	0.0	0.0	0.0	0.0	72.3	13.9	11.1	2.7	0.0	0.0
6	27.7	13	88.0	8.0	2.0	2.0	0.0	0.0	58.4	11.5	8.5	8.5	3.1	10.0
7	18.8	20	68.5	11.0	8.2	5.5	4.1	2.7	47.2	19.7	12.1	8.9	3.5	8.6
8	26.8	21	70.0	13.3	13.3	3.3	0.0	0.6	59.1	19.5	12.2	5.5	0.6	3.1
9	100.0	?	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Necrotic Ring Spot														
1	34.5	38	83.9	10.7	4.7	0.0	0.7	0.0	76.7	14.8	5.3	2.5	0.0	0.7
2	24.6	19	77.5	12.7	7.0	1.4	0.0	1.4	61.3	23.0	8.8	4.6	0.5	1.8
3	29.2	9	57.2	23.8	0.0	9.5	0.0	9.5	70.6	21.6	3.9	3.9	0.0	0.0
5	16.7	4	63.6	27.3	0.0	9.1	0.0	0.0	41.8	21.8	9.1	7.3	1.8	18.2
6	26.6	16	75.0	16.7	0.0	6.2	2.1	0.0	61.4	22.7	10.6	4.5	0.0	0.8

[a] L = planting distance between trees. (See figure 3.)

and subsequent surveys (Fig. 4). New yellows infections can appear at any of the specified distances, but the frequency with which they do is not proportional to the available opportunities but rather drops off markedly as the distance from possible sources increases (Fig. 4). Records for individual orchards, with a few variations in details, also tend to conform to the general rule. It may be concluded therefore that yellows tends to spread more readily to adjacent trees than to the more remote ones. However, in spite of this tendency, 47.8, 39.5, and 51.0 per cent of the healthy trees at present adjacent to diseased trees in orchards 1, 3, and 4,
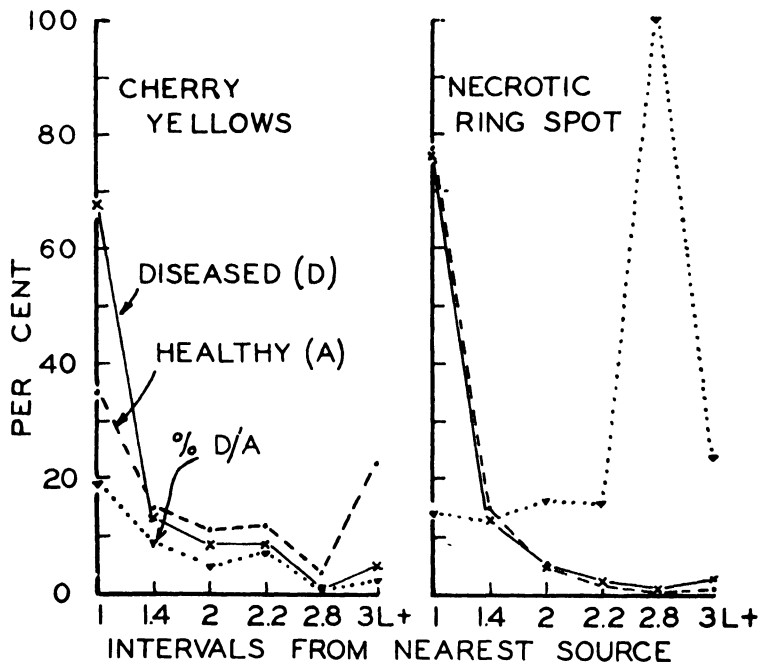
FIG. 4. Percentages of "healthy" trees (A) available for infection, percentages of new cases (D) and the percentage of healthy trees (D/A) becoming diseased, at each interval from the nearest source.

respectively, have occupied that position for three or more years. It would thus appear that transmission of yellows by natural root grafts (2), though a possibility, is not a common occurrence. The evidence obtained from the analysis of distribution of spread, taken as a whole, supports the view that cherry yellows is disseminated in the orchard mainly by a vector, the movements of which, besides being influenced by wind, are habitually restricted to single trees, but which may migrate occasionally from one tree to the next and less frequently to more distant ones. It seems reasonable also to conclude that the vectors are neither numerous nor prolific, otherwise a greater frequency of tree to tree migration and consequently a more rapid increase of the disease could be expected. It might be pointed out that some species of leaf hopper and aphids could fulfill the

necessary requirements. A somewhat similar conclusion was reached by Keitt and Clayton (7) who, after an entirely different approach to the problem, have reported the probability that leaf hoppers (Cicadellidae) may be vectors. Investigations are now in progress to determine and establish these points.

If the analysis of spread of necrotic ring spot is taken at its face value, the results indicate that this disease has a wider range of spread than yellows. The curve showing the percentage of new cases at each distance from the nearest diseased tree is virtually identical with the corresponding curve for "healthy" trees available for infection (Fig. 4). This in itself suggests that the frequency of new infection at any distance from source is directly proportional to the number of opportunities available. The same conclusion may be drawn from the curve for the percentage of "healthy" trees becoming diseased in each category. It may be noted in passing that the 100 per cent point reached at 2.8 L in this curve is purely fortuitous since there were only two trees in the category and both of them happened to become infected. Before accepting the above-mentioned conclusion and its corollary that different types of vector are responsible for the spread of necrotic ring spot and yellows, it should be remembered that the same distribution in the orchard could be obtained with a more limited range of speed, providing a number of apparently healthy but actually diseased trees were scattered through each of the orchards. Since there is good evidence, already submitted, for believing this condition to be fulfilled, it is considered to be the major part of the explanation for the apparent distribution of spread of necrotic ring spot. Thus there is at present as much reason for concluding that the means of dissemination of necrotic ring spot are very similar to, if not the same as, those of yellows, as there is for concluding that they are different.

<div align="center">OVERLAPPING INCIDENCE</div>

The fact that yellows has seldom if ever been transmitted unaccompanied by indications of the presence of necrotic ring spot has given rise to the idea either that the two sets of symptoms may be phases of the same disease or that symptom expression of yellows in sour cherries may occur only when the tree is infected by a mixture of the yellows and necrotic-ring-spot viruses. Accordingly the frequencies with which each disease appears alone and in combination and of the orders in which infection with each disease occurred in mixture have been determined (Table 7). In most of the orchards more than half of the total number of trees known to be diseased were infected with necrotic ring spot alone and considerably less than half appeared to be infected with yellows alone. Also, about two-thirds of the trees known to have necrotic ring spot did not have yellows while half the trees that had yellows were not known to have necrotic ring spot. Since a substantial proportion of the trees showing symptoms of only one disease had been infected for four or more years, it would

TABLE 7.—*Frequency of association of yellows and necrotic ring spot and of order of appearance of infections*

Combination	Orchard 1 No.	Orchard 1 Pct.	Orchard 2 No.	Orchard 2 Pct.	Orchard 3 No.	Orchard 3 Pct.	Orchard 5 No.	Orchard 5 Pct.	Orchard 6 No.	Orchard 6 Pct.	Total No.	Total Pct.
N.R.S. only	92	23.9	161	83.1	39	66.1					292	45.8
N.R.S. first	135	35.2	15	7.7	9	15.2					159	25.0
In same year	13	3.4									13	2.0
Y first	6	1.6	3	1.5	3	5.1					12	1.9
Order not known	13	3.4	1	0.5	1	1.7					15	2.4
Y only	125	32.5	14	7.2	7	11.9					146	22.9
Total diseased trees	384	100.0	194	100.0	59	100.0					637	100.0
N.R.S. only	92	35.5	161	89.4	39	75.0	24	63.2	60	84.5	376	62.7
N.R.S.—Y	167	64.5	19	10.6	13	25.0	14	36.8	11	15.5	224	37.3
Total N.R.S.	259	100.0	180	100.0	52	100.0	38	100.0	71	100.0	600	100.0
Y only	125	42.5	14	42.4	7	35.0	16	53.3	39	78.0	201	47.3
Y—N.R.S.	167	57.5	19	57.6	13	65.0	14	46.7	11	22.0	224	52.7
Total Y	292	100.0	33	100.0	20	100.0	30	100.0	50	100.0	425	100.0

appear that necrotic ring spot is not necessarily a phase of yellows. That yellows can occur without necrotic ring spot is not deducible from the percentage of trees showing only yellows symptoms because necrotic ring spot may be masked in many or all such cases. But it is indicated as a possibility by the small percentage of cases in which yellows symptoms were observed for two or three years before the appearance of severe or acute symptoms of necrotic ring spot. The evidence on the whole favors the theory that the two diseases are caused by different viruses and can occur independently but the possible effect of necrotic ring spot on symptom expression of yellows has yet to be completely determined, if and when the casual viruses are separated.

Because of the potentially wide distribution of necrotic ring spot at planting time, it is to be expected that new yellows infections in orchards would, in the ordinary course of events, occur more frequently in trees already affected with necrotic ring spot than in healthy ones. In 26.4 per cent of the cases where necrotic ring spot preceded yellows, acute symptoms of the two diseases appeared in successive years. Some of these cases should perhaps be regarded as simultaneous or coincidental infections with the two viruses, since yellows symptoms are known to be sometimes delayed until the second year after infection. For the same reason, in some of the cases where acute symptoms of both diseases appeared in the same year, yellows infection could have occurred first. Infections with both viruses in the same year could be brought about either simultaneously by the same vector or accidentally by different vectors, but further evidence is necessary to substantiate either conclusion.

SUMMARY

Annual surveys have been conducted in several sour cherry orchards over a period of years to determine the incidence, the distribution of old and new cases, and the rates and conditions of spread of cherry yellows and necrotic ring spot. The problem is complicated by the tendency for the latter disease to become masked in course of time. Analysis of the data indicates that a greater percentage of trees are likely to be infected with necrotic ring spot than with yellows when an orchard is set out, that rates of spread are largely determined by initial incidence and by the relative position of affected and "healthy" trees at planting, and that little fundamental difference in the rates and manner of spread of the two diseases can be expected even when apparent differences are considerable. Cherry yellows tends to spread more frequently to adjacent than to more remote healthy trees and its dissemination appears to be influenced to some extent by prevailing winds. It is suggested that yellows may be carried from tree to tree mainly by an insect vector which only infrequently moves from one tree to another and whose movements at such times may be affected by air currents.

In each orchard surveyed, some trees showed symptoms of only one of

the diseases, some, symptoms of both, and some were apparently healthy. Since the symptoms of yellows appear annually and those of necrotic ring spot may become masked, trees showing only the latter may be presumed yellows-free, but trees showing only yellows symptoms may also have necrotic ring spot. In cases where trees are known to have both diseases, necrotic ring spot most frequently preceded yellows, but sometimes yellows appeared before or at the same time as necrotic ring spot.

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BROWN STEM ROT OF SOYBEAN

WILLIAM B. ALLINGTON AND DONALD W. CHAMBERLAIN [1]

Brown stem rot of the soybean (*Glycine max* (L.) Merrill) was first discovered in 1944 in central Illinois (1). Since that time it has become one of the most important diseases of the soybean crop throughout Illinois and is becoming increasingly important in Indiana, Iowa, Missouri, and Ohio. A short description of the disease has been given by Allington (1). Presley and Allington (3) identified the causal organism as a member of the genus *Cephalosporium*, but made no species identification. It is the purpose of this paper to describe the causal organism and to report the results of current studies on the disease.

SYMPTOMS

The first symptom of brown stem rot usually appears in July or early August in Illinois. At this time infected plants show no external evidence of the disease. When the stems are split longitudinally, however, a brown coloration of the pith and vascular elements is plainly visible, extending upward from the base of the stem (Fig. 1, E). In seasons which are unfavorable for disease development, only the internal stem symptoms may be found. At first the browning extends but two or three inches above the soil line, more prominent at the nodes. Later, under conditions favorable for the disease, the browning becomes continuous throughout the stem. External symptoms of the disease usually appear in late August or the first week in September when a sudden blighting and drying of the leaves is noted. The onset of this phase is so rapid that the farmer often suspects frost damage. The leaf tissues adjacent to the veins remain green while the interveinal portions die (Fig. 1, A). From a distance a badly infected field appears brown as contrasted with the yellow-green of a normally maturing field.

The severity of the final effects of brown stem rot depends somewhat upon weather conditions at the time of harvest. If warm, dry weather prevails, infected soybeans may be harvested with little loss. However, under conditions of low temperatures before harvest time the weakened stems lodge badly and substantial losses occur because of mechanical difficulties in harvesting. When harvest is delayed by unfavorable weather or other factors, losses are greatly accentuated since the weakening of the stems with subsequent lodging continues until harvest.

THE PATHOGEN

Macroscopic Characters. The macroscopic characters of the pathogen are, unless otherwise stated, those observed in 14-day-old cultures on 2 per
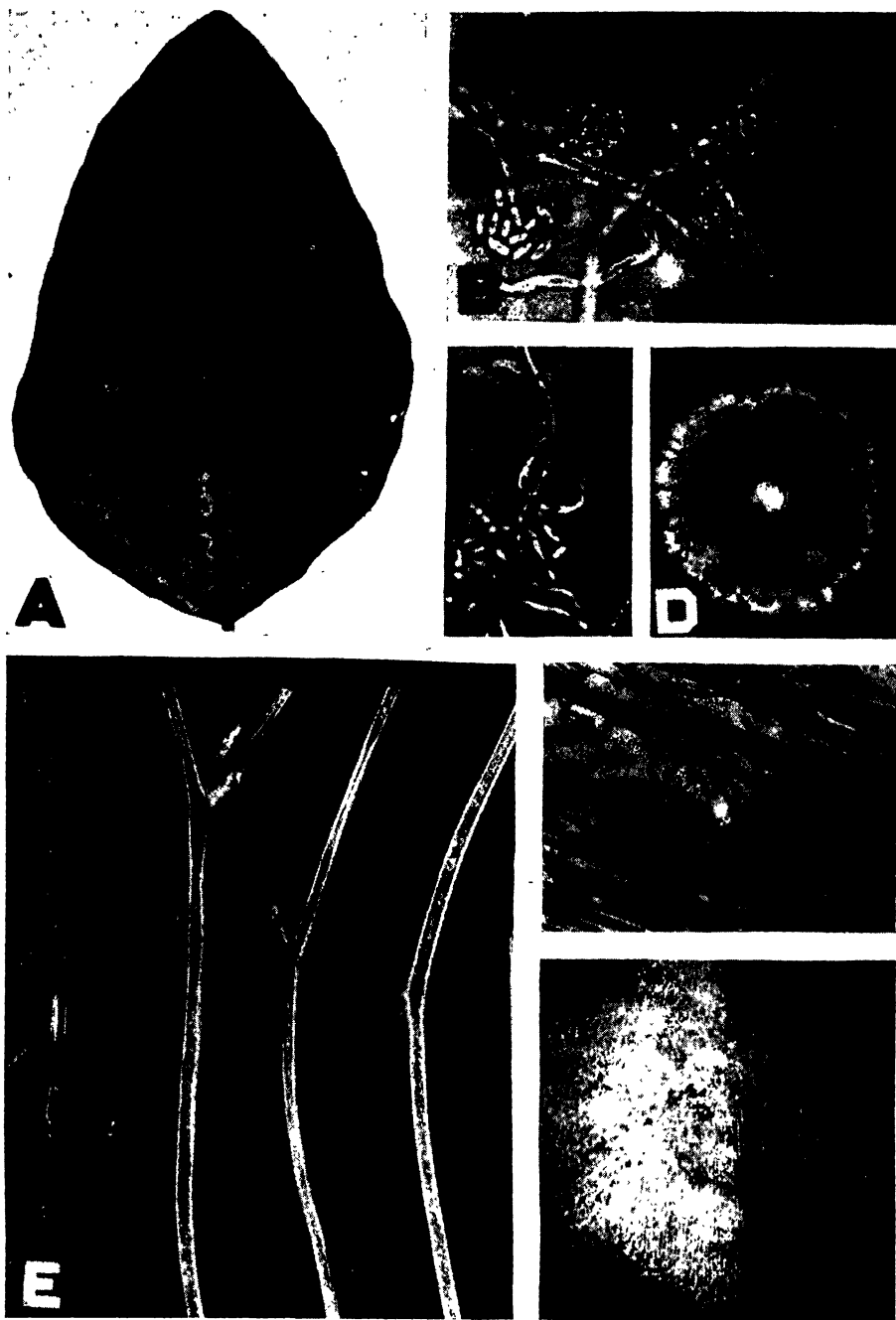
Fig. 1. Symptoms of brown stem rot of soybeans and characters of the causal organism (*Cephalosporium gregatum* n. sp.) observed in pure culture. A. Leaf symptoms observed in late fall. B. Conidial heads in pure culture produced on soybean-stem agar. C. Aggregated conidiophores and conidia observed in pure culture on soybean-stem agar. D. Three-weeks-old culture on potato-dextrose agar. E. Brown coloration in vascular system and pith of naturally infected field plant. F. Longitudinal section of vessel in stem showing fungus mycelium in vessel. G. Aerial view (altitude 600 ft.) taken in mid-September showing sharp line of demarcation between diseased and healthy portions of a soybean field, resulting from crop rotation. Right, diseased; left, healthy.

cent potato-dextrose agar at 20° C. Mycelial growth is slow, producing a flat, dense, approximately orbicular mat, white to putty-colored or gray, with radial folds that become more prominent with age (Fig. 1, D). The margin is variable, but usually finely lobed. In 7- to 14-day-old cultures the mycelial mat is slightly floccose. Through the bottom of the Petri dish the cultures appear smooth, tan with a gray margin, and sometimes slightly concave. The diameter of 14-day-old cultures, growing from 4-mm. mycelial disks at 20° C., varies from 2.0 to 2.4 cm.

On soybean-stem agar[2] the organism produces a thin, spreading, transparent mycelial growth, inconspicuous by reflected light. In cultures 3–4 weeks old concentric rings can be seen by transmitted light. On this medium the diameter of 14-day-old cultures varies from 2.3 to 2.9 cm.

Microscopic Characters. The hyphae are hyaline, septate, branched, and 1.2–4.7 μ in diameter. In cultures more than ten days old the hyphae frequently form aerial or decumbent fascicles. On soybean-stem agar the conidiophores are usually short, 4–15 μ long, but occasionally reach 25 μ in length. They are hyaline, continuous or septate, simple or branched, straight or club-shaped. They may be produced at any point on the fertile hyphae, usually appearing in groups (Fig. 1, C). When borne at the tip of a fertile hypha, the conidiophore is not well differentiated. Conidia are produced in succession at the apex of the conidiophore to form an irregular head, and are non-catenulate (Fig. 1, B). When the heads are produced in close proximity they aggregate to form large, irregular spore masses, either on the surface of the agar or on aerial hyphae. These spore masses disintegrate instantaneously upon contact with water unless they have undergone considerable drying as would be the case in old cultures. The conidia are non-septate, ovoid to elliptical, hyaline, 1.7–3.4 × 3.4–7.6 μ. Spore heads or conidiophores have not been observed in or on soybean plants, but the conidia have been found in the exudate taken from infected cut stems to which water pressure had been applied. The conidia produced within the soybean stems are typically larger (3.4–4.3 × 6.8–9.4 μ) than those produced in pure culture. Conidia germinate by a single germ tube from one end or occasionally by two germ tubes, one from each end of the spore.

Cultural Studies. The organism has shown no noticeable genetic variation in pure culture over a period of three years. Sectoring or changes in pathogenicity have not been detected, and all the isolates taken from naturally infected plants for a period of three years appear to be identical. When cultures were started from finely macerated mycelium, variations in color from white to dark brown occurred but subsequent transfers yielded only the normal type.

Sporulation does not occur on potato-dextrose agar in sufficient quantities to be detected except by a critical examination of washings made from such cultures. By this method a few spores sometimes can be found, as reported

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[2] Soybean-stem agar was prepared by adding an extract of 25 gm. macerated green soybean stems in 100 ml. water to 1 liter of 2 per cent agar.

by Presley and Allington (3). A large number of vegetable decoctions in 2 per cent agar, with and without dextrose added, were tested as media for satisfactory sporulation. Conidia were produced on string-bean agar, rice-polish agar, cucumber agar, and soybean-stem agar. The addition of 2 per cent dextrose to the above media inhibited sporulation. Since spore production was best on the soybean-stem agar, it was used almost exclusively for the microscopic studies of the pathogen involving spore production. Spores appeared on soybean-stem agar cultures after five days at 20° C.

The effect of temperature upon growth rate was studied on potato-dextrose agar and soybean-stem agar. The minimum temperature for growth is below 8° C., the optimum 22°–24° C., and the maximum above 30° C. The optimum temperature for sporulation in culture is between 15° and 20° C., definitely below the optimum for mycelial growth. Sporulation has never been observed at temperatures above 28° C.

Germination of conidia was observed after 24 hours in distilled water and in concentrated soybean-stem juice at 15°, 18°, 20°, 21°, 24°, 25°, 27°, and 30° C. The highest percentage of germination was 88 in soybean-stem juice. The optimum temperature range was 21°–25° C. At 15° C. germination was 0–2 per cent; at 30° C. it was 0–9 per cent.

Host Range Studies. Extensive tests have not been made in studying the host range of the brown stem rot organism. A few species of cultivated plants were inoculated, however, by making stem punctures near the base of the stem and inserting mycelium. This method has never failed to result in heavy infection of soybean. The following species were tested for susceptibility: tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), red kidney bean (*Phaseolus vulgaris* L.), castor bean (*Ricinus communis* L.), mung bean (*Phaseolus aureus* Roxb.), pea (*Pisum sativum* L.), sunflower (*Helianthus annuus* L.), red clover (*Trifolium pratense* L.), and corn (*Zea mays* L.). The disease developed only in mung bean. Not only were symptoms absent in the remaining species, but in repeated trials the organism was never reisolated from the inoculated plants. In all cases soybeans were used as a check on the technique used and infection and recovery of the pathogen always occurred.

Comparison of the Cultural and Parasitic Characters of Cephalosporium acremonium Corda and the Brown-Stem-Rot Organism. Reddy and Holbert (4) have reported the pathogenicity of *Cephalosporium acremonium* Corda to corn in the Midwest, consequently the brown-stem-rot fungus was compared very closely with it.

In culture the two organisms are distinct. *Cephalosporium acremonium* on potato-dextrose agar produces a white, floccose, orbicular mycelial mat that later develops a typical pinkish tinge. As stated earlier, the brown-stem-rot fungus produces a flat, dense, radially folded mat of putty-colored mycelium. On potato-dextrose agar *C. acremonium* sporulates profusely in 5–7 days, while the soybean pathogen produces few or no spores. On soy-

bean-stem agar both organisms produce a thin, transparent mycelial growth and sporulate abundantly. The rate of growth of *C. acremonium* is about twice that of the soybean parasite on both media. Detailed differences in morphology of the two species are described under the section on taxonomy.

Thirty different corn hybrids were inoculated in the seedling stage with the two organisms by the stem puncture method. Although no definite disease symptoms were produced by either organism, the corn was definitely susceptible to *Cephalosporium acremonium*. This organism grew within the plants and was reisolated after six weeks from 75 per cent of the plants inoculated. On the other hand, the soybean parasite was not reisolated from the corn in repeated attempts. Seventeen soybean plants (var. Bansei) were inoculated with *C. acremonium* and a similar number with the soybean parasite. *C. acremonium* produced no symptoms and was only recovered in 2 per cent of the platings of tissue at the points of inoculation. The soybean parasite caused the majority of the plants to develop typical brown stem rot symptoms and 90 per cent of the platings were positive.

Taxonomy.[3] The brown stem rot pathogen apparently belongs in the genus *Cephalosporium* Corda of the Moniliaceae. No other organism of this genus has previously been described as a parasite on soybean. The description of this organism does not seem to conform to the descriptions of the *Cephalosporium* species listed by Buchanan (2) and Saccardo (5, 6, 7, 8, 9, 10, 11). *C. acremonium* Corda, a parasite on corn in Illinois (4), resembles the soybean pathogen somewhat on the basis of spore shape. However, the conidia of *Cephalosporium acremonium* $(1-1.8 \times 2.5-6\,\mu)$[4] are appreciably narrower and shorter than those of the soybean parasite. *C. acremonium* produces well-separated conidial heads on erect conidiophores. On favorable media the soybean pathogen produces short, straight or club-shaped conidiophores, singly or more frequently in groups. This latter behavior results in the most outstanding characteristic of the fungus, suggesting the specific name. The heads, produced in close proximity to each other, aggregate to form large, irregular masses of spores at the surface of the agar or slightly above it. This differs markedly from anything described for any known member of the genus *Cephalosporium*. The brown stem rot pathogen, however, appears to be more closely related to that genus than to any other. Accordingly, the name *Cephalosporium gregatum* n. sp. is proposed.

Cephalosporium gregatum n. sp.

Mycelial mat dense, flat, orbicular, white, later becoming putty-colored or gray, with conspicuous radial folds; sterile hyphae hyaline, septate, branched, $1.2-4.7\,\mu$ broad, often forming aerial fascicles or tufts; fertile hyphae usually $1.2-2.0\,\mu$ broad, aerial or decumbent; conidiophores short, usually $4.2-15\,\mu$ but occasionally up to $25\,\mu$ in length, simple or occasionally branched,

straight or club-shaped, usually continuous but occasionally septate, sometimes produced singly but usually in clusters, either terminally or along the fertile hyphae; conidia ovoid to elliptical, 1.7–3.4 × 3.4–7.6 μ in culture, up to 4.3 × 9.4 μ in the host, hyaline, continuous, capitate, not catenulate; conidial heads irregular, aerial or decumbent, usually aggregating to form mound-like masses of spores close to or at the surface of the medium.

Habitat: In roots and stems of living soybean plants (*Glycine max*) and in soils in Illinois, Indiana, Iowa, Kentucky, Missouri, and Ohio, U.S.A.

Mycelium in culturis tegeticulam compactam decumbentemque formans, orbicularem, primo albam denique griseam colore, in rugas radiantes conspicuas plicatam; hyphis sterilibus hyalinis, septatis, ramosis, 1.7–4.7 μ crassis, saepe in fasciis aeriis protrudentibus; hyphis fertilibus aeriis vel decumbentibus, plerumque 1.2–2.3 μ crassis; conidiophoris brevibus, plerumque 4.2–15 μ, aliquando ad 25 μ longe, simplicibus vel ramosis, rectis vel clavatis, continuis tamen nonnumquam septatis, aliquando extremitatibus saepius ullis intermediis locis hypharum fertilium in caespitibus locatis; conidiis ovoideis vel ellipticis, 1.7–3.4 × 3.4–7.6 μ in culturis, ad 4.3 × 9.4 μ in hospitibus, hyalinis, continuis, capitatis, non catenulatis; capitibus irregularibus, aeriis vel decumbentibus, plerumque grumulos conidiorum summis mediis formatibus.

Habitat in radicibus et caulibus plantarum vivarum (Glycine max) et in terris in Illinois, Indiana, Iowa, Kentucky, Missouri, et Ohio, U.S.A.

HOST-PARASITE RELATIONS

It has been repeatedly demonstrated that the pathogen can enter the host through artificial wounds at the base of the stem. In the greenhouse, plants growing in naturally infested soil have become infected without artificial wounding. In steamed sand, infection was obtained by pouring inoculum around the roots or by dipping the roots into the inoculum before transplanting. It appears that in fields in Illinois penetration occurs early in the summer, perhaps 3–4 weeks after planting, and that the main and lateral roots are the most common points of entry. Microscopic examinations at weekly intervals throughout the growing season have consistently revealed the presence of the organism in the vessels of the main root prior to stem invasion. The amount of mycelium in the vessels varies from a few strands to a solid mass of hyphae sometimes occupying almost the entire lumina of the vessels (Fig. 1, F). The fungus appears to migrate through the walls between the vessels. In actively growing plants it is predominantly a vascular parasite and is found only occasionally in the pith. The organism progresses upward through the vessels of the stem, lateral branches, and petioles, and can be found near the tips of the plants at maturity. In mature plants mycelium is found in the pith more abundantly than at any previous stage of plant development.

It does not seem likely that the organism overwinters in stems. When diseased plants were dried and kept in the greenhouse for 3 months, it was

not possible to recover the organism. Numerous attempts to isolate the fungus from overwintered stems in the field have likewise failed. It has been demonstrated, however, that the fungus can exist for more than a year in moist soil stored in the greenhouse. The slow growth habit of this organism has not permitted isolation, as yet, directly from the soil. Plants grown in infested soil under the proper environmental conditions readily become infected and thereby supply a reliable criterion for the existence of the parasite in the soil. Seed from diseased plants has been plated out repeatedly but no indication has been found that the organism is seed borne.

Effect of Temperature on Disease Development. The effect of soil temperature on infection and development of the disease was studied in the greenhouse. Naturally infested soil was placed in earthenware containers and maintained at the following temperatures: 10°, 15°, 20°, and 25° C. Six pots with at least four seeds planted in each pot were used at each temperature. The experiment has been repeated twice, using four pots instead of six as in the first test. The air temperature of the greenhouse varied from 15° to 26° C. during the tests. Only a trace of infection was detected at each of the temperatures. The plants were grown to within two weeks of maturity in most cases. Although these results are inconclusive, it is believed that the disease is not particularly responsive to different soil temperatures.

The effect of air temperature on the incidence and development of the disease was also studied in the greenhouse. Three controlled air-temperature chambers at 15°, 21°, and 27° C. were used. In several tests the Lincoln and Bansei varieties of soybeans, six to ten inches tall, were artificially inoculated near the ground level by stem punctures. Small amounts of mycelium from a non-fruiting culture on potato-dextrose agar were inserted into the punctures. After two to three weeks at 15° C. air temperature, the internal browning of the stems was evident 8–10 inches above the point of inoculation, and typical leaf symptoms of the disease were observed. At 21° C. progress of infection was almost inhibited and at 27° C. no spread of the fungus was observed. Platings from these stems two to three inches above the point of inoculation failed to yield the organism. Isolations within one inch of the point of inoculation in the plants held at 21° and 27° C. usually were positive. Lowering of the temperature to 15° C. after incubation at 21°–27° C. always resulted in a severe attack of the disease within two weeks.

In two tests inoculum from soybean-stem agar bearing abundant conidia was compared with inoculum from potato-dextrose agar bearing no conidia by means of basal-stem-puncture inoculations. In plants held at air temperatures below 21° C. the inoculum bearing conidia caused typical symptoms and death of the plants before symptoms were evident in the plants inoculated with the potato-dextrose agar cultures. Noninoculated plants in the same environment remained healthy. All the plants held above 21° C.

remained symptomless regardless of the type of inoculum used. However, when the temperature was lowered below 21° C., all the inoculated plants soon developed symptoms of the disease. The time required for symptoms to develop in this case was noticeably different with the two inocula used. It was evident that the presence of spores was a major factor in the time required for development of the disease in plants held at low temperatures from the time of inoculation.

Conidia were produced in the vascular system of naturally or artificially infected plants held at temperatures below 21° C. This was demonstrated by forcing water through the cut stems under considerable pressure and examining the exuded water microscopically. Also by this method suspensions of spores from pure cultures have been passed through sections of healthy stems, indicating the possibility that spore production and movement may be of importance in the spread of infection within the plant.

EFFECT OF CROP ROTATION ON THE INCIDENCE OF
BROWN STEM ROT IN ILLINOIS

The external plant symptoms in the later stages of the brown stem rot are so conspicuous that one may readily identify them by aerial observations. The over-all brown color caused by the dead leaves contrasts sharply with the yellow-green of normally maturing fields. Consequently, in September of 1946 an aerial survey of brown stem rot was made. One of the most conspicuous characters of the disease from this vantage point was that certain fields were only partly diseased, and a sharp line of demarcation separated the diseased and healthy portions of the fields (Fig. 1, G). Such fields were located on a detailed map while in the airplane and the lines of demarcation roughly sketched. The farm was later visited by car, the diagnosis of brown stem rot verified, and the farmer interviewed in regard to history of the diseased and healthy portions of his field.

Without exception, the information gained by this method indicated a crop rotation effect upon the incidence of the disease. Each of these fields served as two experimental plots, providing an accurate history could be obtained. Within a very short period of time (2 weeks) 35 such fields were investigated. Completely reliable histories were available on 15 of these fields for a period of at least four years. A four-year rotation of corn, soybeans, oats, and clover controlled the disease completely on the farms studied, while portions of the same fields which in previous years had been farmed separately and therefore had different crop histories were severely infected. Typical examples of rotation histories on severely diseased fields were as follows: (1) corn, soybeans, corn, soybeans; (2) soybeans, corn, corn, soybeans; or (3) clover, corn, soybeans, soybeans. Continuous cropping to soybeans is of course very rare, but a few cases were found and invariably severe stem rot was present. In many cases the healthy portion of the field was growing its first crop of soybeans. The disease was not found where

a previous crop of soybeans had not been grown within the previous three years. On the basis of these observations, it seems reasonable to conclude that one method of controlling the disease in Illinois is to practice a four-year rotation.

DISCUSSION

Brown stem rot of soybean is becoming of great importance in the areas of intensive soybean production in midwestern United States. The organism is probably indigenous in view of the fact that it is non-parasitic to the other common crops of the area, that it is soil-borne and definitely affected by the crop rotations practiced in recent years, that it can grow saprophytically, and that the disease is unreported elsewhere. Soybean culture in the area affected is relatively new, although intensive; in view of the present knowledge, one may believe that its sudden appearance and devastation is primarily the result of cropping practices of the area.

In view of the accumulated knowledge to date, one might logically expect the disease to spread northward from its present location with continued cultivation of the soybean crop in northern areas. It is doubtful, in view of the critical low temperature requirements, that the disease will become of importance in the southern soybean-producing areas of the United States.

The lack of response to soil temperatures and the striking response to air temperatures noted in this study are of special interest. These results are somewhat correlated with the behavior of the organism in pure culture. Mycelial growth was optimum around 22°–24° C., while spore production was optimum below 20° C. The presence of abundant spores in infected plants growing at low temperatures, the decidedly greater potency of inoculum bearing spores, and the demonstration that the spores move readily through the vascular system suggests an explanation for the rapid development of the disease in the upper plant parts under cool conditions. Further work will be necessary to prove this hypothesis, however. It is possible that the spread of the organism, which in pure culture, at least, is a very slow grower, within the upper plant parts is dependent upon the conidia that may move up the stem in the transpiration stream.

Infection from the soil in the roots and basal stem portions apparently proceeds very slowly under high temperature conditions of mid-summer, but with the occurrence of a few cool days in the autumn enough conidia are produced to spread rapidly up the stem. This possibly would also explain the rapid onslaught of the disease in the fall.

SUMMARY

The *Cephalosporium* causing brown stem rot of soybeans is described and the name *Cephalosporium gregatum* n. sp. is proposed.

A limited survey revealed no hosts other than soybean and mung bean for this pathogen.

Air temperatures are of critical importance in the development of the disease, but soil temperatures were not found to be of significance. Air temperatures below 21° C. were essential for the rapid spread of the organism up the stem and the development of typical leaf symptoms. A possible explanation of the mechanism of this reaction is advanced, based upon the production of conidia within the vascular system.

Detailed studies of disease epidemics, aided by aerial observations, revealed a close relationship between the incidence of this disease and crop rotations practiced in the areas most affected. A crop rotation system which provides for three successive annual crops other than soybeans between soybean plantings is recommended for control of brown stem rot in Illinois.

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SUMMARY OF COOPERATIVE EXPERIMENTS ON TREATMENT OF FLAX SEED[1]

J . E . MACHACEK[2] AND F . J . GREANEY[3]

This cooperative investigation, commenced under the auspices of The American Phytopathological Society's Committee for Coordination in Cereal and Vegetable Seed Treatment Research, was begun in 1944. Initially embracing trials at 8 stations in the United States and 2 in Canada, the project has expanded to include trials at 21 stations. The locations of these stations and the names of the cooperators are listed in table 1.

The present report, covering the 3-year period 1944–46, deals with cooperative tests of some standard and some new seed disinfestants or protectants applied to "sound" and to "fractured" flax seed (Fig. 1). The nature of the injury to the seed suggests that the materials tried could function in these tests only as protectants. In view of the diverse conditions under which the tests were made, it is hoped that the findings reported here will be applicable over a wide area of this continent.

MATERIALS AND METHODS

The "sound" seed used in these experiments was derived from reasonably healthy crops of Royal flax in which the plants were threshed by passing them between the rollers of an ordinary clothes wringer. The amount of fracturing in this seed ranged from 0 to 4 per cent. Seed containing 31 to 53 per cent of "fractured" seeds was obtained by threshing parts of the above crops with a steel stationary thresher.

The preparations applied to flax seed in the present tests were as follows: New Improved Ceresan (5 per cent ethyl mercury phosphate), Semesan Jr. (1 per cent ethyl mercury phosphate), Dubay 1452F (7.7 per cent ethyl mercury p-toluene sulfonanilide), Spergon (98 per cent tetrachloro para-benzoquinine + 1 per cent phosphate), and Arasan (50 per cent tetramethyl thiuramdisulphide). They were generally applied at the rates recommended by their manufacturers. Several rates of application of New Improved Ceresan, however, were tested, chiefly for the purpose of comparing the rate ($\frac{1}{2}$ oz. per bu.) recommended in the United States with that ($1\frac{1}{2}$ oz. per bu.) recommended in Canada.

Each year, field plans and seed for the entire experiment were prepared at Winnipeg and distributed to the various cooperators. The seed was put up in paper coin-envelopes (one envelope for each row) at the rate of 300 seeds per envelope for seed germination tests, and in lots equivalent
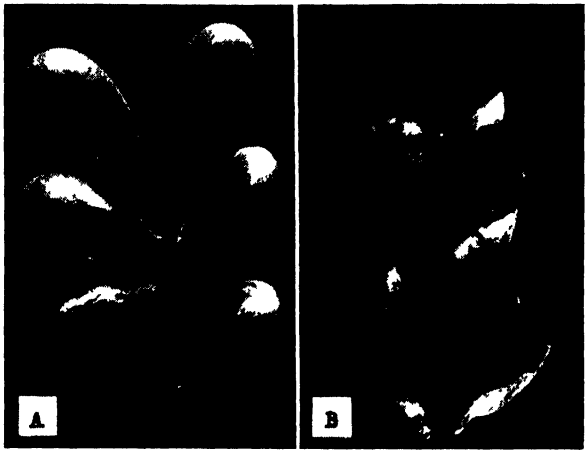
FIG. 1. Kinds of seed used in cooperative seed treatment experiment. A. Sound seed. B. Fractured seed.

to a seeding rate of 28 lb. per acre for yield trials. Before the seed was dispatched to the cooperators, envelopes containing seed treated with the same preparation were tied together and wrapped with wax paper to reduce to a minimum the escape of volatile constituents present in some of the preparations.

A uniform, randomized, split-block plan of experimentation (4 replicates) was used at all stations. One-half of each block was sown with sound seed and the other half with fractured seed. The different treatments were randomized within each half-block. In the seed germination

TABLE 1.—*Location of tests in the cooperative seed treatment investigation with flax*

State or Province	Location of test	Cooperators
New York	Geneva	W. F. Crosier
Iowa	Ames	C. S. Reddy
Wisconsin	Madison	H. L. Shands, D. C. Arny
Minnesota	St. Paul	M. B. Moore
South Dakota	Brookings	W. F. Buchholtz, C. N. Nagel
North Dakota	Fargo	W. E. Brentzel
Montana	Bozeman	R. H. Bamberg
Washington	Pullman	C. S. Holton
Manitoba	Winnipeg	F. J. Greaney, J. E. Machacek
	Morden	W. R. Leslie, W. J. Breakey
	Brandon	M. J. Tinline, W. H. Johnston
	Gilbert Plains	A. H. Parker
Saskatchewan	Indian Head	J. G. Davidson
	Melfort	M. J. McPhail, W. T. Burns
	Scott	A. G. Kusch
	Swift Current	A. W. Platt, B. C. Jenkins
	Saskatoon	H. W. Mead
Alberta	Lethbridge	W. D. Hay
	Lacombe	F. H. Reed, G. E. Delong
	Beaverlodge	W. D. Albright, E. C. Stacey
	Edmonton	A. W. Henry

tests, carried on in Canada and the United States, individual plots consisted of single rows, 10 ft. long and 1 ft. apart. In the yield trials, all of which were in Canada, the plots consisted of 4 or 5 rows, 18 ft. long and 9 in. apart. The 5-row plots were used at a few stations where it was considered advisable to obtain information on both germination and yield, one row of the plot being pulled to obtain a germination count for the plot. To furnish information on yield, the middle 2 rows of the remaining 4 rows in such plots were harvested, after 9 in. from the ends of each row had been discarded. In yield trials where 4-row plots were employed, the same general harvesting procedure was followed.

Each year, the experimental data obtained at cooperating stations away from Winnipeg were forwarded to the authors for compilation and analysis. A report on the whole project was subsequently dispatched from Winnipeg to each cooperator. Also, in 1944 and 1945, interim reports were published in the Plant Disease Reporter.[4, 5]

EFFECT OF SEED FRACTURING ON GERMINATION AND YIELD

It was shown earlier[6] that the percentage germination of fractured flax seed is generally lower than that of sound seed, and that the decrease in germination is roughly proportional to the percentage of fractured kernels present in the seed. It was shown, too, that seed fracturing, unless a high percentage of seeds is affected, does not always adversely affect the yield. It appeared from observations on growth that the increased tillering of flax plants in thin stands tended to compensate for loss of seedlings resulting from decay of fractured seed in the soil. In the present experiment, conducted over a wider area, a somewhat similar result was obtained. Although the percentage germination of fractured flax seed was decidedly lower than that obtained with sound seed at most of the cooperating stations, at only a few did seed-fracturing adversely affect the yield.

EFFECT OF VARIOUS SEED TREATMENTS ON GERMINATION

The data summarized in table 2 show that, in 1944 and 1945, none of the seed treatments had any appreciable effect on the germination of sound seed. In 1946, however, some of the treatments increased the germination of such seed by a significant amount—a fact indicating the possible presence of pathogenic organisms on the seed.

Fractured seed responded favorably to seed treatment in each year of the 3-year experimental period. In 1944, a significant increase in germination was obtained for all the seed treatments employed with the exception of New Improved Ceresan applied at the low rate of ¼ oz. per bu. In 1945, significant increases in germination were obtained for all the treat-

4 Greaney, F. J.   Co-operative flax seed treatment tests in 1944.   U. S. Dept. Agr., Plant Dis. Reptr. Suppl. 159: 215–219.   1945.
5 ——————.   Co-operative flax seed treatment tests in 1945.   U. S. Dept. Agr., Plant Dis. Reptr. 30: 113–119.   1946.
6 Machacek, J. E. and A. M. Brown.   Threshing-injury to flax seed in Canada.   Scient. Agr. 25: 601–625.   1945.

TABLE 2.—*Effect of various seed treatments on the percentage germination of sound and fractured Royal flax seed*

Treatment	Dose (oz./ bu.)	Sound seed[a]			Fractured seed[b]		
		1944[c]	1945	1946	1944[c]	1945	1946
New Improved Cercsan	0.25	61.3	65.5	58.6	28.5	32.2	35.3
	0.50	62.0	67.0	60.6	29.4	36.7	41.0
	1.00	60.0	66.8	63.0	33.9	41.2	49.0
	1.50	60.5	66.8	61.3	33.4	45.4	51.0
	2.00	61.6	66.4	64.3	36.4	46.9	49.6
	3.00	60.9			35.3		
Semesan Jr.	2.50	60.0			30.9		
Dubay 1452F	0.50		66.5	61.0		34.5	40.6
	1.00		65.8	62.6		37.1	43.0
Spergon	1.50	58.4	65.8	54.6	29.5	31.3	38.0
	3.00	60.0	65.5	58.3	30.2	33.8	36.3
Arasan	1.50	60.8	65.9	60.0	29.3	36.5	44.3
	3.00	60.0	66.0	59.0	32.0	39.8	39.6
Control		60.0	65.2	54.6	25.1	30.4	33.0
Significant difference (5 per cent level)		D.N.S.[d]	D.N.S.	3.5	3.9	2.5	5.1

[a] Threshed with ordinary clothes wringer. There were no fractured kernels present in the seed used in 1944 and 1945. In 1946, four per cent of the kernels were fractured.

[b] Threshed with stationary thresher. Content of fractured kernels was 31 per cent in 1944, 48 per cent in 1945, and 53 per cent in 1946.

[c] Mean of data from 11 stations in 1944 and 13 stations in 1945 and 1946.

[d] D.N.S. = Difference not significant.

TABLE 3.—*Effect of various seed treatments on the yield of sound and fractured flax seed*

Treatment	Dose (oz./ bu.)	Yield (bu./acre)					
		Sound seed[a]			Fractured seed[a]		
		1944[b]	1945	1946	1944[b]	1945	1946
New Improved Cercsan	0.25	22.2	28.4	22.5	22.2	22.3	19.2
	0.50	21.0	27.6	21.4	22.0	23.6	20.4
	1.00	22.4	27.7	21.1	23.2	26.0	21.1
	1.50	22.5	28.6	21.5	22.0	27.2	21.4
	2.00	23.1	28.5	23.4	23.0	26.9	21.5
	3.00	22.4			21.2		
Semesan Jr.	2.50	21.6			21.5		
Dubay 1452F	0.50		28.3	21.7		25.4	20.1
	1.00		27.9	22.6		24.9	20.8
Spergon	1.50	20.6	27.9	22.0	21.2	21.6	19.5
	3.00	22.0			22.5		
Arasan	1.50	22.0	27.2	21.9	21.2	24.0	20.3
	3.00	22.9			23.0		`
Control		21.5	28.4	21.7	21.2	20.6	17.9
Significant difference (5 per cent level)		D.N.S.[c]	D.N.S.	D.N.S.	D.N.S.	2.3	2.1

[a] For percentages of fractured kernels in seed, see footnotes under table 2.

[b] Mean of data from two stations in 1944, four stations in 1945, and 10 stations in 1946.

[c] D.N.S. = Difference not significant.

ments tested except New Improved Ceresan (¼ oz. per bu.) and Spergon (1½ oz. per bu.). In 1946, the results were about the same as in 1945 except that Spergon did not improve germination even when used at the rate of 3 oz. per bu. At some stations (Pullman, Washington; Fargo, North Dakota; and Geneva, New York) the response to seed treatment was usually very slight.

EFFECT OF VARIOUS SEED TREATMENTS ON YIELD

None of the treatments applied to sound flax seed increased the yield, even in 1946 when germination of sound seed was improved considerably by seed treatment (Table 3). With moderately-fractured seed, such as was used in 1944, the observed increases in yield were not statistically significant, but with somewhat more severely-fractured seed, such as was used in 1945 and 1946, increases were significant. These increases were relatively large in 1945, but comparatively small in 1946. There was a good deal of variation in the amount of benefit from treatment, but unlike the situation observed with seed germination, there was no station at which seed treatment continually appeared to be without worth.

From table 3, it would appear that New Improved Ceresan, when applied to fractured flax seed at the rate of 1½ oz. to 2 oz. per bu. increased the yield to the greatest extent. Only in 1945, however, was the yield at these dosages significantly higher than that obtained with the dosage (¼ oz. per bu.) generally recommended in the United States, while in no instance was it significantly higher than the yield from the dosage (1 oz. per bu.) generally recommended for the drier parts of Western Canada. Dubay 1452F and Arasan also significantly increased the yield but not so much as New Improved Ceresan. As only one or two dosages of these treatments were employed it was impossible to determine if they were the optimal ones. Treatment of flax seed with Spergon did not bring about any increase in yield.

SUMMARY AND CONCLUSIONS

Cooperative tests of various seed treatments, applied to sound and fractured flax seed, were carried on during a 3-year period over a wide area of Western Canada and in the northern part of the United States. In all, 13 stations in Canada and 8 stations in the United States participated.

The data obtained from the experiment indicate that treatment of flax seed, particularly when a considerable proportion of the seeds are fractured, is a sound practice. It was found that treatment of seed damaged by fracturing generally increased germination and occasionally increased yields. The data suggest also that, when New Improved Ceresan is used, it should be applied to flax seed at a higher dosage than ½ oz. per bu. An increase of this dosage to at least 1 oz. per bu. is indicated.

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NONSUSCEPTIBLE HOSTS AS CARRIERS OF WILT FUSARIA[1]

G. M. ARMSTRONG AND JOANNE K. ARMSTRONG[2]

INTRODUCTION

Since 1932, stems of many thousands of plants have been cultured on agar plates to isolate wilt Fusaria. At first the cotton plant chiefly was used, but in recent years, a variety of plants has been included, especially in the numerous inoculation experiments. In a study of the pathogenicity and cultural characteristics of the cotton-wilt organism, Armstrong *et al.* (3) reported that a *Fusarium* could be obtained from a fairly high percentage of inoculated cotton plants of a resistant variety that showed no discoloration of the internal tissues. It was assumed that most of the Fusaria recovered were the cotton-wilt fungus, and limited tests indicated that this was generally true. When inoculations of various species of plants were made in considerable numbers, as for example the sweet potato inoculated with the cotton-wilt fungus, Fusaria were likewise obtained from a fairly high percentage of the stalks when cultured on agar plates, although either no or slight external or internal symptoms of wilt were apparent. These results led to the assumption that some of the wilt Fusaria, supposedly restricted in their host ranges, are parasitic[3] on many plants that show no symptoms of disease. Experimental data to support this assumption are presented.

METHODS

Plants were grown in soil, in solution-culture, and in steamed sand. The inoculation procedures in soil using a wheat-oats mixture (3) and in solution-culture (1) have been described. For the steamed-sand cultures, the plants were grown in 2-gal. glazed pots with a 2-cm. hole laterally at the bottom for drainage and either of two inoculation procedures was used. In Method A the roots were inoculated by pouring a 3-day-old solution culture of the fungus into holes made around the base of the plants growing

[2] Head, Department of Botany and Bacteriology, and Agent, Division of Cotton and Other Fiber Crops and Diseases, respectively.

[3] Definitions submitted by the Committee on Technical Words. Phytopath. 30: 361–368. 1940. RESISTANCE: "Ability of the host to suppress or retard the activity of a pathogenic organism or virus." NONSUSCEPTIBLE is used in preference to resistant since there are, for example, varieties of cotton which are resistant to the cotton-wilt Fusarium, but cotton is considered nonsusceptible to the sweet-potato-wilt Fusarium. PARASITE: "Organism or virus for which the tissues of another living organism serve as substratum and source of nutrition." The organisms dealt with in this paper are considered parasitic but not pathogenic since there were "either no or slight external or internal symptoms of wilt" when the plants were inoculated with them.

in the pot.   In Method B, the roots of young plants which had been grown in flats of sterilized soil were washed, dipped in the inoculum, and set immediately in a pot of sand.   In both methods a second inoculation was made a week or more later using the procedure in Method A if the plants showed no symptoms of wilt at that time.   Method A is essentially that of Tharp,[4] and Method B is a modification of that described by Wellman (12).

All methods have given approximately the same results.   With the solution-culture technique somewhat more care is necessary to prevent contaminations, although a greater number of plants can be grown in a given area by this method than in soil or sand.   The procedures for preparing and sterilizing the wheat-oats mixture and incorporating it in the soil are time-consuming, and serious difficulties may be encountered with damping-off due to the addition of considerable organic matter unless the soil is kept for two or three weeks before planting.   Certain soils become toxic to plants after steaming which was particularly true of the manganiferous Lloyd soil used in this investigation.   The sand-culture technique was very satisfactory, and Method A was used wherever possible.   Check plants and those not infected upon inoculation grew vigorously notwithstanding the cutting of some roots.

In Methods A and B of the sand-culture technique the inoculum was prepared by culturing the fungus for 72 hr. at 28° C. on the following liquid medium in a flask which was shaken thoroughly three or four times daily to keep the mycelium well dispersed: sucrose or glucose, 2 per cent; $MgSO_4$, 0.003 M; KCl, 0.022 M; KH_2PO_4, 0.1008 M; $Ca(NO_3)_2$, 0.10356 M; $FeCl_3$, $MnSO_4$, and $ZnSO_4$, 0.2 p.p.m. each of cations.   The isolates of Fusarium were maintained on slants of potato-dextrose agar.   A suspension of the fungus for inoculating the liquid medium was made by pouring 10 ml. of sterile water on a fresh slant-culture, loosening spores and mycelium with a sterile needle, and using this at the rate of 2 ml. per 1000 ml. of liquid medium. ˙

The nutrient solution described by Armstrong (1) was used for growing the plants.   Good growth was obtained with all plants except sweet potatoes which required increased iron up to double that indicated.

During the course of the experiments, the usual precautions were taken to prevent accidental contaminations.   Sterilized rubber gloves were worn when making the inoculations for each isolate, and all the necessary glassware and instruments were also sterilized.

Cotton, Cassia (*Cassia tora* L.), okra, soybean, and cowpea were grown in pots of sand and Method A was used in the inoculation experiments. Immediately before planting, the seeds of cotton, Cassia, and okra were immersed in concentrated sulphuric acid for 5 min. and then thoroughly washed in tap water.   Seeds of soybean and cowpea were surface sterilized in a hypochlorite solution.   Twenty seeds were planted per pot evenly spaced in a circle 1 inch from the periphery and ¾ inch deep.   Tap water

[4] Personal communication from Dr. W. H. Tharp, Jr.

was used sparingly until all seedlings had emerged. After about seven or eight days when the plants were usually thinned to ten per pot, nutrient solution was used. The first inoculation was made when the unexpanded first true leaves appeared which was about 10–14 days after planting for cotton but somewhat sooner for the others, depending upon light conditions and temperatures. The second inoculation was made one week later. The inoculation procedure was as follows: 1 hr. after applying the nutrient solution, an inverted Buchner funnel was pressed into the moist sand to a depth of about 1½ inches. Five hundred ml. of the well shaken inoculum was poured into the depression where the roots had been cut. The roots were then covered and after a few hours about 1 quart of tap water was applied to each pot. The same procedure was used for the second inoculation a week later except that when a large number of different isolates was involved, it was more convenient to use a large sterile test tube instead of a funnel for cutting the roots in each pot. Dr. Tharp used 1 qt. of nutrient solution twice daily when the plants began to transpire rapidly but this amount was used only once daily in these experiments with satisfactory results. Large plants, however, frequently required water in addition to the nutrient solution to maintain their turgidity. Once a week tap water was used in place of the solution. The tests with cotton were continued for six to seven weeks, and those with the other hosts for eight weeks.

Method B was used in the experiments with seedlings of tomato, sage, tobacco, snapdragon, mimosa (*Albizzia julibrissin* Duraz), and Mexican clover (*Richardia scabra* St. Hil.). For sweet potato, sprouts or vine cuttings which had been rooted in sand were used, and, if a clear-cut injury was not evident at the base, a small portion of the stem was cut with sterilized scissors before the roots were dipped in the inoculum. Experience has shown that a low percentage of wilt occurs in plants without an injured basal stem. Poole and Woodside (8) stated that infection is almost entirely through the basal end of the plant. After the roots were dipped in a 3-day-old liquid culture of the fungus, five plants were set per pot of sterilized sand and 500 ml. of the inoculum distributed around them. Only 250 ml. was used for sweet potato since a larger amount seemed at times to be somewhat toxic to the tender sprouts and vine cuttings. However 500 ml. of inoculum was applied in the second inoculation. The second inoculation, when necessary, was made one week or more later, depending upon the condition of the plants, by cutting some of the roots with a large test tube placed close to the stem of each plant and distributing the inoculum among the holes made by the tube. At times such definite symptoms of wilt appeared after the first inoculation that a second was clearly unnecessary. The sweet-potato plants showed either very definite or no external wilt symptoms in a relatively short time, consequently, in the later tests they were not grown so long as the other plants but were removed for plating at about four weeks.

Plants in all of the experiments were removed from trays or pots when

definite external symptoms of wilt were apparent. A piece of the basal stem was washed in running water, after which a 1-inch section just above the soil line was cut, dipped in 95 per cent ethyl alcohol, immersed for 5 min. in a calcium hypochlorite solution (BK)[5] containing 1667 p.p.m. of available chlorine, and plated on 1.8 per cent water agar. This concentration of hypochlorite was used on all types of tissue and the procedure was standard throughout these experiments. Plants which had not wilted at the end of an experiment were usually treated similarly.

Efficacy of Hypochlorite as a Surface Disinfectant

The assumption has been that the wilt Fusaria recovered from the stems of plants which showed no wilting were actually inside the stems. If the Fusaria were only attached to the outside surfaces and the hypochlorite failed to kill the organisms, the results as reported would have no significance. Even though the stems did not contain a wilt *Fusarium,* other fungi frequently grew from the pieces. It was important to know whether the hypochlorite disinfects the stem surfaces and, particularly, if it kills the wilt Fusaria which might adhere to the base of the stems. The efficacy of the hypochlorite as a surface disinfectant in the concentration used throughout this work, viz., 1667 p.p.m. of available chlorine, was checked by dipping 24 1-inch, thin, wooden sticks in each of two suspensions of different Fusaria. The sticks were surface sterilized and plated according to the standard procedure described for the stem sections. The suspensions of a sweet-potato-wilt and a cotton-wilt *Fusarium,* were made by adding 10 ml. of sterile water to a test-tube slant and either scraping the surface with a needle or shaking the tube in which the cotton plug was replaced by a sterile rubber stopper. The scraping methods loosened pieces of agar which had to be removed by straining through a thin layer of cotton, before complete sterilization was obtained. An examination of both of the suspensions showed that pieces of mycelium and the three spore forms were present in abundance. A check consisted of ten sticks dipped in each of the suspensions, with sterile water substituted in the subsequent steps for the alcohol and the hypochlorite, to ascertain if the spores and the mycelia had been washed off in the dipping process. All of the sticks that had been treated with hypochlorite were sterile after incubation at 28° C. for two weeks. In a few days, the Fusaria grew abundantly on 18 sticks of the check or unsterilized series. These results indicate that the hypochlorite in the concentration used in these experiments will kill the wilt Fusaria on ordinary surfaces.

EXPERIMENTAL RESULTS

Inoculations of Sweet Potatoes with a Cotton-Wilt Isolate and with Other Fusarium Isolates from Healthy Sweet Potatoes

In the summer of 1940, sweet potatoes growing in a solution culture in the greenhouse were inoculated with the cotton-wilt fungus. Both the

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[5] The commercial product, BK, was used.

Nancy Hall and Porto Rico varieties were grown in the first experiments, but since no differences in the susceptibility of the two were noted, thereafter only the Porto Rico variety was used. The plants showed no external symptoms of wilt but a *Fusarium* similar to the original in cultural characteristics was obtained from 70.3 per cent of the plants when stem sections were plated (Table 1, Exp. 1, a). Since the bases of the stems in the check or uninoculated tray (Exp. 1, b) showed no Fusaria when cultured on agar plates, it was inferred that the sweet potato might be a carrier of the cotton-wilt fungus and thus show no symptoms of wilt. It is generally assumed that many of the wilt Fusaria will survive in the soil for long periods without the presence of the usual host plants. If they occur as benign invaders in hosts other than those considered susceptible, another method of their survival will have been revealed.

In other experiments in which sweet-potato plants were inoculated with various wilt organisms, Fusaria were repeatedly recovered from some of the healthy plants in the check trays or pots (Table 1, Exp. 2, a–b; 4, b) as well as from some of the plants which had been inoculated. Thus it seemed that plants produced from bedded potato roots only rarely were fusarium-free, although they were healthy and showed no external symptoms of wilt.

During the course of these experiments, uninoculated plants of cotton, okra, tobacco, tomato, soybean, cowpea, two weeds, and snapdragon were being grown in solution cultures or in steamed soil or sand. Stem sections of many of these were plated, but *Fusarium oxysporum* Schl. according to the system of Snyder and Hansen (11) was seldom observed. Thus it appeared that the technique used was not responsible for the infection of the uninoculated sweet-potato plants but that unblemished sweet potatoes might contain Fusaria which grew into the sprouts when the potatoes were bedded. The sweet-potato wilt pathogen, *F. oxysporum* f. *batatas* (Wr.) S. and H. (5) is known to pass from the bedded root into the sprouts. It was necessary, therefore, to study more carefully the Fusaria occurring in the healthy roots and sprouts so that the results obtained in the pathogenicity tests could be evaluated. Accordingly, certified sweet potatoes were obtained and 20 of these were treated in a hypochlorite solution for 10 min. Under aseptic conditions in a culture-room, pieces of internal tissue from near the stem end, the basal end, and the middle of each potato were plated on water agar. A Fusarium isolate was obtained from only two potatoes and these isolates were used separately to inoculate plants in trays of solution culture in order to determine their relationship to the sweet-potato-wilt fungus. The combined results are given in table 1, experiment 4, a. Likewise comparable trays of plants (Table 1, Exp. 3) were inoculated, respectively, with four isolates from the healthy check plants (Table 1, Exp. 2, a–b). A tray of uninoculated plants (Table 1, Exp. 4, b) was included. The plants for these tests were obtained from certified potatoes which had been surface disinfected before being bedded in the greenhouse in steamed sand in a disinfected bench.

There were no external wilt symptoms in any plant, though some slight internal discoloration was noted in from 12.5 to 22.8 per cent of the plants. This may not be significant, however, since Fusaria were recovered from two to three times as many plants as those which showed internal discoloration viz., from 39.6 per cent of the check plants and from 54 per cent of one of the inoculated groups. Various investigators have noted darkening of the vascular bundles of the stems of plants affected with Fusarium wilts and many have used it as a criterion of infection. Cook (4) has shown, however, that browning of the bundles of tomato stems is an inadequate criterion of infection.

The isolates used for these inoculations were grown on rice, Irish-potato plugs, and potato-dextrose agar along with some wilt pathogens from sweet potato, cotton, and tobacco. The microscopic and cultural characteristics of the isolates indicated that all belong to the section Elegans as described by Wollenweber and Reinking (13).

The results obtained in the previous experiments, especially from plating sections of both the healthy potatoes and the plants produced from the sprouts of these potatoes, seemed to indicate more clearly that sweet potatoes harbor Fusaria internally which are not the sweet-potato-wilt pathogen. It should be noted that Fusaria were obtained from 10 per cent (2 of 20) of the potatoes but from 39.6 per cent (Table 1, Exp. 4, b) of the plants grown from them. One would not expect too close a correlation of such results since only three small pieces of each potato were cultured. The entire potato is bedded and there is a tendency for the sprouts to be produced in groups at the ends.

Since a further study of a larger number of potatoes was desirable, three lots of potatoes were secured, one each from Spartanburg, S. C., from Clemson, S. C., and from the Edisto Experiment Station, Blackville, S. C. Seventy potatoes from each lot were immersed in hypochlorite solution for 6 min., and, following the procedures previously described, a piece of the internal tissue about an inch from each end of every potato was plated on water agar. Fusaria were obtained from 14.3, 7, and 7 per cent, respectively, of the three lots. A discoloration of the vascular ring of some of the potatoes, varying from fairly distinct to slight, was noted but no correlation existed between this condition and the isolation of Fusaria from the potatoes. For example, in the Spartanburg lot, Fusaria were obtained from only five of the 20 potatoes showing discoloration, however, five other potatoes appearing perfectly normal also contained Fusaria.

The potatoes of the three lots from which Fusaria were not obtained were bedded separately by lots in clean beds of steamed sand in the greenhouse. Those from which Fusaria were obtained were bedded similarly. Sprouts from the former were considered as fusarium-free, while those from the latter were considered as possibly diseased. Sprouts from the various lots were grown separately in trays of solution culture, but the results have been combined in table 1, experiments 5, a–b. Even if some of the

original potato roots harbored Fusaria and some apparently did not, the plants from both sources contained a rather high percentage of Fusaria without showing symptoms of wilt.

The plants in table 1, experiment 6, were inoculated with a *Fusarium* obtained from one of the healthy Edisto potatoes, but no wilting occurred.

Since the cotton-wilt organism is present in some of the fields of the Edisto Experiment Station where sweet potatoes are grown it was thought that some of the Fusaria obtained from healthy potatoes might be the cotton-wilt fungus. Accordingly, the isolates from the healthy Edisto potato (Table 1, Exp. 6) was used to inoculate two trays containing 173 cotton plants of a wilt-susceptible variety but no wilting occurred.

TABLE 1.—*Results of inoculations of sweet potato with a cotton-wilt Fusarium and with other Fusaria obtained from healthy sweet potatoes*

Exp. No.	Origin of isolate	No. Plants	Medium for growing plants	Percentage of plants with symptoms of wilt Internal discoloration[e]	Recovery of fungus, per cent
1a	Cotton	37	Solution	24.3	70.3
b	Uninoculated check	30	do	0.0	0.0
2a	do	148	do	6.8	55.4
b	do	10	Soil	0.0	20 0
3	Healthy potato plants[a]	197	Solution	22.8	47.2
4a	Healthy potato roots[b]	100	do	18.0	54.0
b	Uninoculated check	48	do	12.5	39.6
5a	Uninoculated[c]	107	do	12.1	65.4
b	Uninoculated[d]	87	do	36.7	62.1
6	Healthy potato roots	145	do	2.1	60.7

[a] Four trays, each with a different isolate.
[b] Two trays, each with a different isolate.
[c] Potatoes which showed no fungus on plated tissues; sprouts from these grown without inoculation.
[d] Potatoes which showed a Fusarium on plated tissues; sprouts from these grown without inoculation.
[e] There were no external symptoms of wilt in any of the plants.

Recovery of the Cotton-Wilt Fusarium from Sweet Potato and Snapdragon

After numerous observations indicated that wilt Fusaria invaded non-susceptible hosts, an attempt was made to isolate some of the Fusaria from these hosts and, by inoculations into a susceptible one, to determine whether the wilt organism had been recovered. Therefore, ten sweet-potato plants were grown in two pots of sand and inoculated with the cotton-wilt fungus. No symptoms of wilt were evident but Fusaria were recovered from nine of the ten plants. An isolate from each plant was used to inoculate a pot of cotton, and, as noted in table 2, experiment 1, eight of these proved to be the cotton-wilt fungus. The ninth isolate was not included in the data because it was nonpathogenic to cotton.

Since the cotton-wilt fungus can invade sweet potatoes when inoculations are performed under greenhouse conditions, it was of interest to know if

natural invasion occurs in the field. Disease-free sweet potatoes were bed-
ded in steamed sand and both sprouts and vine cuttings were set in a field
where cotton had died of wilt. Three settings were made from July 24 to
August 1, 1944. Sprouts and vine cuttings were also planted in steamed
sand in the greenhouse to check if any wilt might appear but none occurred
in either the greenhouse or the field. On October 10, sixty-eight plants were
brought to the laboratory for plating and Fusaria were obtained from 61
of these. Some of the isolates did not belong to *Fusarium oxysporum,* so
only those which appeared most promising from 46 of the plants were tested
for pathogenicity on cotton growing in pots of sand. When the isolates
were tested singly or in combinations of two, the results showed that at least
26 sweet-potato plants carried pathogenic isolates of the cotton-wilt fungus,
although there may have been as many as 34 since this was the number in-
volved in the various combinations. The percentage of cotton plants wilt-
ing in the individual pots was in the range from 81.8 to 100 per cent with an

TABLE 2.—*Results of inoculations of cotton with the cotton-wilt Fusarium recovered
from inoculated sweet potatoes or those naturally invaded in the field, and from inoculated
snapdragons*

Exp. No.	Origin of isolate	No. cotton plants inoculated	Percentage wilt	Medium for growing plants
1	Sweet potato[a]	94	97.9	Sand
2	Sweet potato, field[b]	338	95.6	do
3	Sweet potato, field[c]	114	0	do
4	Snapdragon	11	91.7	do

  [a] Eight isolates from 10 plants tested singly. Ninth not included was nonpathogenic.
  [b] Fifty-three isolates from 34 plants tested singly or in combinations of 2 in 27 pots.
  [c] Seventeen isolates from 12 plants tested singly or in combinations of 2 in 10 pots.

average of 95.6 per cent for the series (Table 2, Exp. 2). The isolates from
12 of the sweet-potato plants proved nonpathogenic to cotton (Table 2, Exp.
3). These results indicated that the cotton-wilt fungus can invade sweet-
potato plants in the field as well as by inoculations in the greenhouse, so
there appears to be nothing particularly artificial in the results obtained
in the greenhouse.

Twelve snapdragon plants in solution culture were inoculated with the
cotton-wilt fungus. No wilting occurred but a *Fusarium* was isolated from
two of the plants. The isolates were mixed for the inoculation of cotton and
91.7 per cent of the plants of the latter wilted as shown in table 2, experi-
ment 4.

Recovery of the Tomato-Wilt Fusarium from Sweet Potatoes and Mimosa

Ten sweet-potato plants growing in sand were inoculated with a tomato-
wilt *Fusarium* but showed no symptoms of wilt in eight weeks. Fusaria
were obtained from all plants, but only the isolates from eight were used
in four pairs to inoculate a total of 20 Pennred tomato plants growing in
pots of sand. Since all the tomato plants wilted, it is evident that the to-
mato-wilt *Fusarium* was recovered from the sweet potatoes.

One of the 13 mimosa plants inoculated with a tomato-wilt *Fusarium* showed a dark streak which extended 3 inches upward in the basal region of the stem although the plant was vigorous and showed no external symptoms of disease. Two Fusarium isolates obtained from this stem were pathogenic to tomato.

Recovery of the Sweet Potato-Wilt Fusarium from Cotton

Fifty-six cotton plants of the Rowden variety growing in a tray of culture solution were inoculated with a sweet-potato-wilt isolate. There were no external symptoms of wilt but Fusaria were recovered from 12.5 per cent of the stems. Three of the recovered isolates when used to inoculate sweet-

TABLE 3.—*Results of inoculations of sweet potato with the sweet-potato-wilt Fusarium recovered from cotton which was inoculated in the greenhouse or naturally invaded in the field*

Exp. No.	Sweet-potato isolate recovered from	No. sweet-potato plants inoculated	Percentage wilt	Medium for growing plants
1a	Cotton[a]	117	100	Solution
b	Cotton[b]	30	100	Sand
2a	Cotton, Resistant, field[c]	40	100	do
b	Cotton, Resistant, field[d]	45	0	do
c	Cotton, Susceptible, field[e]	70	97.1	do
d	Cotton, Susceptible, field[f]	55	0	do
3a	Cotton	5	0	do
b	Cotton[g]	20	100	do
c	Cotton[h]	20	100	do
d	Cotton[i]	20	100	do

[a] Three isolates, each used separately.
[b] Same isolates as a, used separately in sand.
[c] Eleven isolates used singly or in combination in 8 pots.
[d] Thirteen isolates used singly or in combination in 9 pots.
[e] Twenty-one isolates used singly or in combination in 14 pots.
[f] Thirteen isolates used singly or in combination in 14 pots.
[g] Four isolates from wood in basal inch of stem, each used separately.
[h] Four isolates from bark in basal inch of stem, each used separately.
[i] Four isolates from wood of tap root, each used separately.

potato plants grown in solution culture and in sand caused all plants to wilt (Table 3, Exp. 1, a–b). This was interpreted as evidence that the sweet-potato-wilt fungus can penetrate the cotton stem and exist there without producing wilt. To ascertain if this fungus will invade cotton in the field, seed of the wilt resistant variety 4-in-1, and the susceptible variety Rowden, were planted in a field where sweet-potato plants had died of wilt. Eighteen days after planting, 32 and 34 seedlings, respectively, of the resistant and susceptible varieties were pulled, and a short basal section of each stem was plated on water agar, using the method previously described. Seventeen isolates of *Fusarium* were obtained from the Rowden plants and 20 from the 4-in-1 plants. Sixty-three days after planting, the remaining plants were pulled and treated as above. None of the plants showed any external symptoms of wilt yet 18 Fusaria were obtained from 59 of the 4-in-1 plants,

and 25 from the 61 Rowden plants.   No effort was made to identify all of the Fusaria obtained but only the cultures which appeared to be *Fusarium oxysporum* were used in pathogenicity tests.

The difficulties of making pathogenicity tests with all of the isolates were somewhat reduced by testing some of them in combinations, as is shown in table 3, experiment 2, a–d.   At least 8 and possibly 11 of the isolates from the resistant 4-in-1 cotton proved to be pathogenic to sweet-potato plants while 13 of the isolates were nonpathogenic.   At least 14 and possibly 21, of the isolates from the susceptible Rowden cotton were pathogenic while 13 of the isolates were nonpathogenic.   These results indicate that cotton in the field was invaded by the sweet-potato-wilt *Fusarium* present in the soil and that the invasion of the plants in the greenhouse was not dependent upon special conditions.

Recovery of the Sweet-Potato-Wilt Fusarium from Inoculated Cassia, Mexican Clover, Okra, Sage, Tomato, and Soybean

Numerous inoculation experiments with the sweet-potato-wilt *Fusarium* on various hosts have been conducted.   In many cases *Fusarium oxysporum* was recovered from the stems of plants which showed no symptoms of wilt. When some of these isolates were tested on sweet-potato plants growing in sand, it was discovered that the original sweet-potato-wilt organism had been recovered.   The results of the inoculations of Cassia, Mexican clover, okra, sage, tomato, and soybean, together with the experimental procedures are summarized in table 4.   It will be seen that this wilt organism was recovered from all of these hosts since sweet-potato plants wilted when inoculated with some of the isolates.

TABLE 4.—*Inoculation of several species of plants with the sweet-potato-wilt Fusarium and the subsequent recovery of the fungus as shown by tests on sweet potato*

Host inoculated with sweet-potato-wilt Fusarium	Inoculation data			Recovery data		
				Isolates tested on sweet potato plants growing in sand		
	Medium for growing plants	No. plants inoculated and cultured	Percentage recovery of Fusaria	No. isolates tested	No. plants	Percentage wilt
Cassia	Steamed Soil	17	64.7	2	15	100
Mexican Clover	Solution	14	14.3	2	5	100
					5	0
Okra	Sand	11	90.9	9[a]	15	100
Sage	do	9	66.7	1	5	60
Tomato (Bonny Best)	do	12	25.0	1	5	100
Soybean (Yelredo)	do	12	75.0	2[b]	5	100

[a] In combinations of 3.
[b] Two isolates combined.

Isolation of the Sweet-Potato-Wilt Fusarium from Naturally Invaded Mexican Clover

Mexican clover growing in hills with wilted sweet-potato plants was examined and slightly darkened streaks were noted in the lower region of the stems of several plants. Fusarium isolates were obtained from these sections when they were plated. Several of the isolates were nonpathogenic to sweet potato but one proved to be the wilt organism since it killed inoculated sweet-potato plants grown in sand. Thus a weed which showed no external symptoms of wilt was a carrier of the fungus.

In table 4, it will be noted that the sweet-potato-wilt fungus was isolated from two weeds, Cassia and Mexican clover, and four cultivated plants. Since the fungus was recovered from plants of widely divergent families, it seems reasonable to suppose that it might invade plants of other families. All of the plants were exposed to a rather heavy inoculum but this is apparently not a critical factor since the fungus was recovered from both naturally invaded cotton (Table 3) and Mexican clover.

Recovery of the Tobacco-Wilt Fusarium from Inoculated Cassia, Cotton, Okra, Sage, Mexican Clover, Cowpea, and Soybean

A *Fusarium* isolated from flue-cured tobacco (Tobacco isolate No. 1) causes wilt of flue-cured tobacco, Burley tobacco, and sweet potato. In other experiments not related to this study, it had been noted that a *Fusarium* was rather generally recovered from many plants inoculated with this fungus, even though they were healthy and showed no external symptoms of wilt. The apparent entrance of this fungus into a wide range of plants led to its inclusion in these tests in which okra, Cassia, Mexican clover, cotton, cowpea, soybean, and sage were inoculated. Another tobacco-wilt isolate (Md. Mudd) was tested only on soybean.

The recovered Fusaria were tested for pathogenicity on sweet potato rather than tobacco since the former is easier to grow and also wilts quickly, usually in one to two weeks, whereas it may take as many months to get similar results with flue-cured tobacco.

Detailed data are not presented in tabular form due to limitations of space but the tobacco-wilt *Fusarium* No. 1 was recovered from Cassia, cotton, okra, sage, cowpea, soybean, and Mexican clover, since sweet-potato plants wilted when inoculated with the isolates. The "Md. Mudd"-tobacco *Fusarium* was also recovered from soybean.

Location of Wilt Fusaria in Tissues of Plants Without Wilt Symptoms

Nearly all of the nonsusceptible plants from which wilt Fusaria have been obtained showed neither external nor internal symptoms of wilt except for a slight discoloration of the woody tissues in a small number of cases. Since wilt Fusaria readily invade the vascular systems of susceptible hosts and to some extent those of resistant varieties, it might be supposed that they may be found also in the bundle regions of nonsusceptible hosts.

To locate the fungus in the plant the following experiments were performed. The wilt-susceptible Rowden variety of cotton was grown ten plants per pot in ten pots of steamed sand. After inoculation with a sweet-potato-wilt isolate, the plants grew vigorously, without symptoms of wilt and reached an average height of 36.5 inches in eight weeks when the experiment was terminated. Another pot of ten uninoculated plants was used as a check. As the plants were removed, all stems and roots were washed thoroughly and allowed to dry before being cut into sections. Three sections of the stem were used, viz., a 1-inch section of the base at the soil line, B, the 12th inch from the soil line, C, and the last inch at the tip, D. Later the bark was removed from the wood of the basal and middle sections but not from the tip section. A 1-inch section of the tap root about ½ inch below the basal section of the stem was also removed and later the bark was separated from the wood. Approximately 1-inch sections of three of the side roots were used without separation of bark and wood. All of the sections were treated in hypochlorite solution by the standard procedure and plated on water agar. If the three side roots per plant are considered a unit, 880 units of tissue from 110 plants were surface disinfected, plated, and then observed for the growth of organisms. Where bark and wood were separated, the entire stem or root section was dipped in 95 per cent alcohol and immersed in hypochlorite solution for 5 min. It was then placed in a sterile Petri dish with the lid slightly raised while bark and wood were separated with sterile scalpel and tweezers. The bark was transferred directly to water-agar plates. The woody portion, however, was again put in the hypochlorite solution for an additional 5 min. to obviate the possibility that organisms from the bark might have been transferred to the wood. A transfer of a Fusarium isolate was made to agar slants from each of 190 pieces of tissue on the plates and all but one, were similar in cultural characteristics such as color, type of mycelial growth, lack of sclerotia, etc., to the original sweet-potato isolate used for the inoculations. At least one of these typical cultures was obtained from some section of all but one of the inoculated plants. A portion of the data on the recovery of apparently pure cultures of the wilt fungus is shown in table 5. A typical culture was obtained from root sections of 65 plants and from the basal inch of stem of 62 of the same plants. The largest number of cultures was obtained, however, from either the bark or wood of the basal inch of stem of 96 plants. A typical *Fusarium* was recovered from the bark only of 78 and from both bark and wood of 18 of the basal sections of these plants. The extensive histological studies by other workers with numerous wilt pathogens on susceptible and resistant varieties of their host plants have shown the penetration of various host tissues, especially the xylem and cortex. This study of plated tissues indicates that the sweet-potato-wilt pathogen may also penetrate the above mentioned tissues of the nonsusceptible cotton plant. Penetration of the xylem of the nonsusceptible hosts is not established by the procedures which were used, but the definite darkening of the xylem

region in mimosa, Mexican clover, some of the other plants, and sweet-potato roots, indicates that this may be a possibility. On the other hand, the recovery of a typical sweet-potato *Fusarium* from three to four times as many cortical sections as from the woody sections of cotton indicates that most of the invasion of these plants may be cortical.

An additional 48 Fusaria which were similar in appearance to the wilt fungus were observed on the plates but were not transferred to agar slants. *Fusarium moniliforme* Sheld. appeared on 56 pieces. Some of the more common organisms growing in the plates were species of *Trichoderma, Penicillium, Alternaria, Macrosporium,* and bacteria. *Trichoderma* occurred frequently on root sections and was also found twice on wood sections 12 inches up the stem. It will be noted that the sweet-potato-wilt *Fusarium* was not recovered from either bark or wood of any 12th-inch stem section. A single culture of *F. moniliforme* was recovered from the bark of a 12th-inch stem section and no other Fusaria were found at this height in either bark or wood. Forty-four per cent of the 12th-inch stem sections were sterile and bacteria occurred on most of the others. Similar results were obtained with the tip section. With the exception of *F. oxysporum,* the organisms which occurred in the inoculated plants also were found in the check or uninoculated plants. In addition, *F. roseum* (Lk.) S. and H. was found in pieces of bark of three stems and in two pieces of the wood of the tap root of check plants. Rudolph and Harrison (9) found this organism[6] in the seed and in steles of healthy mature cotton stems 6 inches above the soil line.

Thirteen of the Fusarium isolates were tested for pathogenicity on sweet-potato plants growing in pots of sand. Nine of the isolates were from wood sections, five of these from the basal inch of stem and four from the tap root. The other four isolates were from the bark of the basal inch of stem. All but one isolate, which was different culturally from the others, proved pathogenic to sweet potato as is seen in table 3, experiments 3, a–d. These results showed that the sweet-potato-wilt fungus occurred in both bark and wood of cotton plants but that it was not found 12 inches up the stem in a period of eight weeks. The cotton-wilt fungus usually can be found in the tips of inoculated susceptible cotton plants in an equal length of time. Except in this experiment with cotton, no attempt has been made to determine very accurately the percentage of plants of a nonsusceptible host from which one of the wilt Fusaria can be obtained but it seems likely that the recovery from inoculated plants in the greenhouse will be higher on account of the greater inoculum potential. This is indicated by the experiments with cotton in which the sweet-potato-wilt fungus was recovered from 12 to 17 per cent of the plants in the field and approximately 99 per cent from those inoculated in the greenhouse. Not all of the isolates from the latter, which comprised a series of 100 plants, were tested for pathogenicity but the close similarity in cultural characteristics of the untested iso-

[6] *F. scirpi* Lamb. and Fautr. according to Wollenweber and Reinking.

lates to those which were tested seemed to indicate a recovery of approximately 99 per cent. An examination of all the data, however, shows that the latter instance is unusual since the recoveries of Fusaria from even the succulent sweet-potato stems were in the range of about 20 to 70 per cent.

The occurrence of Fusaria inside the cotton seed was not considered in this study and no attempt will be made to review the literature, which is given in the papers by Armstrong *et al.* (2) and Rudolph and Harrison (9). The assumption that *Fusarium oxysporum* f. *vasinfectum* (Atk.) S. and H. found in seed reached them through the vascular system is plausible since it is a vascular pathogen. Rudolph and Harrison (9) found *F. moniliforme*, *F. oxysporum,* and *F. scirpi* (*F. roseum*) in receptacles, placentae, and seed of mature plants, which they assume reached these tissues through the vascular system since these fungi were found in sections of the stele 6 inches above the soil line. In the present investigation the fungi were found in 2-month-old stems but they had not grown very far up the stems. *F. oxysporum* was not found in any 12th-inch section, *F. moniliforme* was in a single bark specimen at this height, and *F. roseum* was in a few bark specimens at the same height. The only wood section in which *F. roseum* occurred was in the tap root.

TABLE 5.—*Recovery of Fusaria from sections of roots and stems of 100 cotton plants inoculated with the sweet-potato-wilt fungus*

Section of plant	No. of plants giving an apparently pure culture of the wilt fungus
All sections	99
All roots	65
B (stem)[a]	62[b]
B, bark or wood[c]	96
C and D	0

[a] Stem sections of the 65 plants in which the fungus was found in the roots.
[b] The fungus was found in the bark only of 48 plants and in both bark and wood of 14 others.
[c] The fungus was found in the bark only of 78 plants and in both bark and wood of 18 others.

Recovery of a Mixed Culture of Cotton- and Sweet-Potato-Wilt Fusaria from Cotton

The question next arose as to whether more than one wilt *Fusarium* might invade a plant at a given time, and result in a mixed culture when isolated. For example, would a wilted cotton plant contain both the cotton- and the sweet-potato-wilt Fusaria if these were present where it was growing?

Rowden cotton plants, growing in two pots of sand with 11 and 13 plants respectively, were given two inoculations according to the standard procedures using 250 ml. each of liquid inoculum of a cotton- and a sweet-potato-wilt *Fusarium*. A total of 12 wilted plants was removed from the pots. Isolations of Fusaria were made from the basal stem sections of all but one

of these. Each section was cultured separately on a water-agar plate and several transfers from each were made to potato-dextrose-agar slants. It was not difficult to obtain cultures free from bacteria and other organisms that occasionally grew from the stem if the transfers were made some distance from the section. Since the cotton- and the sweet-potato-wilt isolates selected for the study showed distinct cultural differences, it was possible to distinguish roughly which was predominantly present. The cotton-wilt isolate produced a blue-green pigment in the agar and formed sclerotia of this color, whereas, the sweet-potato-wilt isolate produced only purple tints and no sclerotia. The mycelial growth was also different in some respects.

The isolations on the agar slants which were made directly from the cotton stems appeared in some cases to contain only the cotton-wilt fungus, in others only the sweet-potato-wilt fungus, and in still others a mixture of the two. This variation in composition was again demonstrated when transfers of these isolates were made to potato-dextrose-agar plates and the cultural characteristics of the resulting colonies were noted.

To obtain further evidence as to the nature of the cultures, pathogenicity tests were also made in which ten to seventeen cotton plants per pot and five sweet-potato sprouts per pot were inoculated at the same time with some of the same inoculum from an original isolate of each of eight wilted cotton plants. The results of the cultural studies and the inoculation experiments are presented in table 6. A study of the plate cultures of these isolations indicated that a mixed culture was being used for all but two of the isolates. Numbers 1 and 6 seemed to be pure cultures of the cotton-wilt and of the sweet-potato-wilt Fusaria, respectively, and pathogenicity tests further indicated that this was true. Three of the cultures caused cotton to wilt but not sweet-potato even though the plates showed a mixture of Fusaria. Two isolations caused all plants of both cotton and sweet potato to wilt.

In many plates distinct sectors were produced which resembled one or the other of the Fusaria. If it had not been known beforehand that a mixed culture was being used, these sectors might have been taken to be variants of the cotton-wilt *Fusarium* which was isolated from the wilted cotton plant. The composition of a few of these sectors is shown in table 7. A transfer was made from the middle of the sector to a potato-dextrose-agar slant, and the resulting culture was used to inoculate cotton and sweet-potato plants. It will be seen that none of these sectors was a pure culture of either organism even though it had the cultural characteristics of one or the other of them. One sector that appeared to be the sweet-potato-wilt *Fusarium* also contained that of cotton and the transfer made from this sector apparently was predominantly the latter since no sweet-potato plants wilted. Another that appeared to be the cotton-wilt *Fusarium* failed to produce cotton wilt but caused one of five sweet-potato plants to wilt.

These studies suggest the necessity of using monosporous isolates in cross-inoculation experiments since in this case both the sweet-potato- and the cotton-wilt organisms occurred in some of the cultures obtained from

TABLE 6.—*Identity of isolates from wilted cotton plants inoculated with both cotton- and sweet-potato-wilt Fusaria as shown by cultural studies and pathogenicity tests.*

Isolation from plant no.	Isolate on agar slant resembled original culture from:	Isolate on agar plates resembled original culture from:	Inoculation data	
			Percentage wilt of cotton	Percentage wilt of sweet potato
1	Cotton	Cotton	100	0
2	Cotton	Mixture	100	0
3	Sweet potato	Mixture	100	100
4	Mixture	Mixture	100	100
5	Mixture	Mixture	100	0
6	Sweet potato	Sweet potato	0	100
7	Mixture	Mixture	100	40
8	Cotton	Mixture	100	0

wilted cotton plants. If one had used for the inoculations the cultures obtained from either uniform colonies or sectors, it would have appeared that a presumably pure cotton-wilt culture also caused wilt of sweet potato.

TABLE 7.—*Identity by pathogenicity tests of sectors produced on potato-dextrose-agar plates by two isolates from wilted cotton plants inoculated with both cotton- and sweet-potato-wilt Fusaria*

Plate Culture No.	Sector resembled original culture from:	Plant inoculated	No. plants	Percentage wilt
1 (Produced 3 sectors)	Cotton	Cotton	10	0
		Sweet potato	5	20
	Sweet potato	Cotton	13	100
		Sweet potato	5	40
	Sweet potato	Cotton	10	100
		Sweet potato	5	0
2 (Produced 2 sectors)	Cotton	Cotton	14	92.9
		Sweet potato	5	100
	Sweet potato	Cotton	15	93.3
		Sweet potato	5	100

DISCUSSION

It has been shown by the senior author and others that wilt Fusaria may be found in the stems of resistant varieties of the usual host plants without either external or internal symptoms of wilt. When numerous Fusaria were recovered from the stems of inoculated plants of genera or species that were not recorded as susceptible, tests were made to ascertain how commonly plants might serve as carriers of many of the wilt Fusaria. The results presented in this paper seem to establish the fact that the wilt Fusaria are parasitic on many plants that show no symptoms of disease. There seems to be no doubt that most of the wilt Fusaria can persist in the soil as saprophytes, but the infection of weeds and cultivated plants which have not been classed as host plants must affect their persistence in soils. The percentage of the Fusaria recovered from some of the plants in both green-

house and field was not always high, but in the most detailed experiment in the greenhouse the sweet-potato-wilt fungus was apparently recovered from 99 per cent of the inoculated cotton plants.

In several instances the senior author has observed that the cotton-wilt fungus persisted in the soil for periods varying up to 12 years without an intervening crop of cotton, yet the incidence of wilt at the end of these periods was high. The impression has been gained from the literature that rotations of three or four years for the control of tomato wilt serve to reduce noticeably the incidence of this disease. Is the cotton-wilt organism better able to survive the competition in the soil than is the tomato-wilt fungus, or does the cotton-wilt fungus enter a greater number of nonsusceptible hosts than does the tomato organism and thus facilitate its survival? The question cannot be answered from the data which are presented.

The cotton-wilt fungus was not recovered from bedded sweet-potato roots in the single instance where a test was made, but some sound sweet potatoes carry *Fusarium oxysporum* internally and one is led to infer that wilt Fusaria other than the sweet-potato pathogen may also be present. The occasional occurrence of a wilt where it had not been found previously might conceivably be explained by the use of some preceding root or tuberous crop that carried the organism to which the latter was not ordinarily considered susceptible, as well as by infected seed. In connection with other experiments not considered in this paper, *Fusarium oxysporum* was present in the internal tissues of sound Irish potatoes. The certified potatoes which were used had been grown in a region where Irish-potato wilt is said to be practically unknown and where the certification authorities have a zero tolerance for this disease.

Harter and Weimer (5) injected the sweet-potato-wilt fungus into stem wounds at the surface of the soil using *Solanum melongena* L., *Lycopersicum esculentum* Mill., and other plants. There were no external symptoms of wilt although in most cases there was a blackening of the fibrovascular bundles for a centimeter or so about the wound, from which the fungus could be recovered. Rudolph and Harrison (9) found *Fusarium moniliforme, F. oxysporum, F. scirpi (F. roseum)*, and another unidentified *Fusarium* in cotton seed which the organisms were assumed to have reached through the stele since stem sections 6 inches above the soil line yielded the same Fusaria. We have also found the above mentioned organisms in cotton stems as well as *F. solani* (Mart.) App. and Wr. and some Fusaria as yet unidentified. Smith and Shaw (10) inoculated cotton with a wilt isolate from flue-cured tobacco in four successive tests without the occurrence of typical disease symptoms yet the fungus was recovered from the plants. Padwick *et al.* (7) obtained two Fusarium isolates from field-grown sunn-hemp which failed to cause wilt of sunn-hemp but produced a wilt of pigeon-pea. Yet their experiments showed the pigeon-pea- and sunn-hemp-wilt organisms to be highly specific in causing wilt of each host.

Lutman (6), using the Irish potato plant especially but also several other plants for comparison, attempted to establish the filamentous nature

of the irregular lines which simulate cell walls. These filaments were thought to be Actinomycetes but the isolation of an organism was not attained.

SUMMARY

Healthy sweet-potato roots were shown to carry internally Fusaria of the section Elegans which were not the sweet-potato-wilt fungus. Plants from these roots also contained Fusaria and it is assumed that they came from the parent potato.

Cotton, either inoculated in the greenhouse or grown in a field infested with the sweet-potato-wilt *Fusarium*, was invaded by this organism without showing any symptoms of wilt; and, thus, became a carrier of the fungus. Likewise, no external symptoms of wilt were noted in the plants mentioned below.

Cassia, Mexican clover, okra, sage, soybean, and tomato were inoculated with the sweet-potato-wilt *Fusarium* and the fungus was recovered from the stems as shown by subsequent pathogenicity tests on sweet potato.

The sweet-potato-wilt *Fusarium* was recovered from the weed, Mexican clover, which exhibited no external symptoms of wilt, but grew among wilting sweet potatoes in a field.

Tobacco-wilt Fusaria were reisolated from inoculated Cassia, cotton, okra, sage, cowpea, soybean, and Mexican clover.

Sweet-potato plants were invaded by the cotton-wilt *Fusarium*, either from infested soils in the field or inoculations in the greenhouse.

The cotton-wilt *Fusarium* was reisolated from inoculated snapdragons.

The tomato-wilt *Fusarium* was reisolated from inoculated sweet potatoes and mimosa.

Sections of stems and roots of cotton plants inoculated with the sweet-potato-wilt *Fusarium* were plated on agar to determine the location of the fungus in the host. Bark and wood were separated in the root and stem sections. The fungus was recovered from the wood of the tap root and the basal inch of stem, and also from the bark of the basal inch of stem. It was not recovered from the 12th inch of the stem or higher, indicating that penetration up the stem did not proceed very far in eight weeks.

Mixed cultures of the cotton- and the sweet-potato-wilt Fusaria were used to inoculate cotton, and both Fusaria were reisolated from wilting plants. On many of the agar plates containing the reisolations, distinct V-shaped sectors appeared with characteristics of one or the other of the original cultures. If it had not been known that a mixed culture was being used, these sectors might have been considered variants of the cotton-wilt *Fusarium*. The necessity of using monosporous isolates in cross inoculations with Fusaria to determine host relationships is clearly indicated.

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A NEW VIRUS DISEASE OF BLACKBERRY[1]

NORMAN L. HORN

In 1942 a single strikingly variegated wild blackberry (probably *Rubus allegheniensis*) was observed near Keedysville, Maryland in a raspberry growing district. The symptom pattern (Fig. 1, A) suggested that the plant might be infected with a virus. Cursory examination failed to reveal similarly affected plants within a radius of approximately 200 yards. In 1947 a portion of this plant was removed to the greenhouse for further study.

During this season all blackberry plants within a 200-yard radius were carefully examined for variegational symptoms. No other plants bearing such extensive variegated areas were found. However, ten plants were located in which a few small bright yellow, sharply-defined chlorotic areas (0.3 to 1 cm.) appeared on one or more leaves. In most instances, however, there was only one small chlorotic area on the entire plant.

Three preliminary approach grafts were made to black raspberry plants of the Logan variety to determine the possibility of transmission to this species. Within three to four weeks symptoms appeared on all of the test plants. These consisted of chlorotic patterns similar to those occurring in the blackberry. Two of these raspberry plants were then grafted to healthy wild blackberry plants. In both cases the original pattern was transmitted.

To test transmissibility further, a series of grafts was made in the greenhouse as follows: (1) five healthy blackberry plants (*Rubus allegheniensis* collected near College Park, Maryland) grafted to the diseased blackberry plant; (2) five healthy raspberry plants (hybrid Cumberland × Evans) grafted to the diseased blackberry plant; (3) five healthy blackberry plants grafted to five healthy raspberry plants, which were used as controls. The extent of transmission and date of first symptoms are shown in table 1. Of the ten plants tested, six developed symptoms of the disease in question. Symptoms appeared on five of the plants 30 days after the grafts were made and on one 73 days after grafts were made. Grafts between diseased and test plants in which transmission did not occur were examined and in each case there was a definite organic union.

In every raspberry plant to which the disease was transmitted, first symptoms appeared about two feet distal to the graft. The chlorotic condition then continued to appear progressively toward the growing point. While symptom expression in the blackberry was similar to that in raspberry, in one case chlorotic areas first appeared on a single leaflet immediately below the graft. This was followed by the appearance of chlorosis in adjoining leaves above and below the first infected leaf. In two at-

TABLE 1.—*Graft transmission of a variegation virus from blackberry to blackberry and to raspberry*

Grafts	Plant number	Number of days before symptoms appeared
Healthy raspberry to variegated blackberry	1	30
	2	No symptoms observed[a]
	3	24
	4	29
	5	73
Healthy blackberry to variegated blackberry	1	No symptoms observed[a]
	2	Do
	3	24
	4	Do
	5	25
Controls		
5 Healthy raspberry to 5 healthy blackberry		No symptoms observed[a]

[a] Grafts were made on September 9, 1947 and no symptoms had been observed by December 20, 1947.

tempts the virus was graft transmitted from artificially infected raspberry back to healthy blackberry without alteration of induced symptoms.

The symptom expression varied considerably in individual blackberry and raspberry leaflets, ranging from leaflets having only a few white islands to leaflets which were nearly totally white (Fig. 2). Willison[2] found that leaves of peach seedlings vary greatly in their response to peach blotch with respect to symptom expression. Figure 1 shows the general pattern

FIG. 1. Typical symptom expression in entire leaves of blackberry and black raspberry. A. Symptoms in blackberry. B. Symptoms in black raspberry.

[2] Willison, R. S. Peach blotch. Phytopath. 36: 273-276. 1946.

of symptom expression in typical raspberry (A) and in blackberry (B) leaves.

Raspberry leaf symptoms are shown in figure 2, (A) through (E). Leaflet (A) shows the occurrence of totally green islands which appear darker green than the normal green areas of the leaf within the chlorotic area. Leaflets (B) through (E) illustrate the various positions of the chlorotic areas. Sometimes these occur along the veins (B), along the leaflet margin (C), as small white islands (D), and as scattered white islands in addition to chlorotic areas along the leaf margin (E). These same symptoms occur in blackberry but certain of the less common chlorotic patterns

FIG. 2. Symptoms in leaflets artificially infected by grafting with the blackberry variegation virus. A through E. Symptoms in black raspberry. F through J. Symptoms in blackberry.

(Fig. 2. F, H, and J) occur more frequently than in the raspberry. A single blackberry leaf may contain all the leaflet patterns shown in figure 2 and in raspberry several types may be expressed in a single leaf.

The infected areas of young leaves of both blackberry and raspberry are at first light green, later become yellow, and as the leaves reach maturity, these infected areas become totally white. Blodgett[3] found that there is a similar color sequence of the chlorotic areas in peach leaves infected with peach calico.

Leaf tissue from chlorotic areas was examined for inclusion bodies

[3] Blodgett, Earle C. Peach calico. Phytopath. **34**: 650–657. 1944.

using the trypan-blue technique described by McWhorter,[4] but none were observed.

Transmission by grafting in over 50 per cent of the attempts together with the type of symptoms produced indicate that the causal agent is a virus. A search of the literature has not revealed any reference to such a virus disease in blackberry or raspberry.

No economic importance has been attached to this new virus in blackberry, but since it occurs in the raspberry growing area of Maryland, and since black raspberry is a host of the virus, it may be of potential importance.

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[4] McWhorter, Frank P. Plant virus differentiation by trypan-blue reactions within infected tissue. Stain Technology 16: 143–149. 1941.

MORPHOLOGY AND TAXONOMY OF THE ONION PINK–ROOT FUNGUS[1]

A . M . G o r e n z , J . C . W a l k e r , a n d R . H . L a r s o n [2]

INTRODUCTION

Pink root of onion (*Allium cepa* L.) is incited by a soil-inhabiting fungus which has become endemic in many onion-growing sections of the United States. The causal organism was thought to be a species of *Fusarium* by Taubenhaus and Mally (10), who named it *Fusarium malli* Taub. They based their conclusions on studies of disease development of onion sets planted in previously steam-sterilized, Fusarium-inoculated soil. Five to 15 per cent of the plants in the inoculated soil became infected as compared with 100 per cent in the non-sterilized soil. They reasoned that this difference in infection was probably due to other organisms, especially other species of *Fusarium*, acting in combination with *Fusarium malli*. Sideris (8) named several new species of *Fusarium* which he regarded as capable of inciting the disease but only a small percentage of the plants in his inoculation experiments became infected. In inoculation experiments with various Fusaria, including authentic cultures from Sideris, Hansen (3) failed to produce the disease. He secured positive results from inoculation with a fungus (*Phoma terrestris* Hansen) isolated from diseased roots. With this fungus he obtained production of pycnidia in culture but since only pycnidial primordia were found in infected roots it was concluded that mature pycnidia apparently never occurred on infected onion roots. The pycnidia were described as subglobose, ostiolate, papillate, dark brown to black, carbonaceous, 170 to 350 μ in size, and single or gregarious. The mature pycnidia varied considerably not only in size, but also in shape, papillation, and length and number of ostioles. Some isolates were predominantly pycnidial and others were mostly or entirely mycelial. There was a tendency of isolates to produce pycnidia abundantly when transfers were made with spores and to become mycelial if transfers were made with mycelium. In a later paper (4) Hansen referred to this tendency as "the dual phenomenon." He considered that the changes which occurred in this fungus in culture, as in various other members of Fungi Imperfecti, were not due to mutation but to the development of thalli which had different complements of nuclei. Thus within *Phoma terrestris*, which he reported to have binucleate spores, he separated out three different types by isolating single-spore lines from the original cul-

ture. These were designated as mycelial (M), mycelial-conidial (MC), and conidial (C) types. The M and MC types produced normal pycnidia whereas the C type produced pycnidia with beaks frequently several times as long as the diameter of the pycnidium.

In the present investigation a large number of isolates from various sections of United States were assembled. A morphological study was made of these cultures and the range of morphological variability was determined.

METHODS AND MATERIALS

Most of the isolates of the pink-root organism used in the course of this work were made directly from infected roots. The infected material arrived in excellent condition for making direct isolations when it was packed in moist cotton or in moist soil. In other cases, isolations were made from plants grown on samples of infested soil. A culture of the fungus was obtained from Michigan and one from California.

Recently infected, firm, pink roots were found to be the most desirable for isolation, but success was also had with dry material. The roots were first washed thoroughly in distilled water, then immersed in 1–1000 mercuric chloride solution for three to five minutes or from five minutes to three hours in BK solution made by adding one-half teaspoonful of B-K powder (50 per cent calcium hypochlorite) to 20 cc. water. The roots immersed in mercuric chloride were washed in sterile water and plated; those in B-K solution were removed directly to agar plates.

A wide variety of nutrient media was used with equal success, each plate being acidified with one drop of 75 per cent lactic acid. Thirty plates or more were used for each lot of diseased material. Colonies appeared from the plated roots in three to eight days, depending on the medium and the method of surface sterilization. Pure cultures of the organism were often obtained when dead, diseased roots, containing numerous pycnidial primordia when dug from the soil, were surface sterilized with B-K solution for three hours and plated. In most cases other fungi appeared from the roots in close association with the pink-root organism. The colonies of the latter could be recognized by their relatively slow, compact growth, by the greyish aerial mycelium, and especially by the production of pigment, which varied in color with the type and reaction of the medium but was usually a shade of red. Large numbers of transfers were made from the leading edges of such colonies. The sources of the isolates, their relative virulence and their sporulating capacities are given in table 1.

EXPERIMENTAL RESULTS

Variability of the Isolates

When the isolates were carried on culture media in test tubes and mycelial transfers made about every four months, most of them became mycelial in type. Some of them were kept in MC condition by using the

original bit of inoculum in each successive transfer. No mycelial line, *e.g.*, the hyphal-tip lines of the Colorado, Utah, Louisiana, and Texas isolates, ever reverted to the pycnidial type. The Utah isolate was carried in culture for six years and in this interval never produced pycnidia either in culture or on the host. However, it often produced dark-colored stromata in culture and on the host.

The use of mycelium in transferring did not necessarily result in a loss of the tendency to produce pycnidia abundantly. A pycnidial isolate from Louisiana retained the sporulating character for 18 months when it was kept growing on Petri dishes and transfers were made with mycelium from the leading edge of the colony. There appeared to be a relation between aging and the change from the pycnidial to the mycelial type as mycelial transfers from old plates of pycnidial cultures resulted in my-

TABLE 1.—*The sources of the isolates of the onion pink-root organism, their virulence and their sporulating capacities*

Source of isolate	Virulence	Pycnidial production	
		In culture	On the host
Louisiana	+++[a]	+[b]	+[b]
California	+++	+	+
Colorado	+++	±	+
Texas	+++	+	+
Utah	++	−	−
Illinois	++	+	+
Iowa	++	+	+
Massachusetts	+	−	−
New York	+	+	+
Wisconsin	+	+	−
Michigan	+	−	−

[a] +++ = highly virulent; ++ = moderately virulent; + = mildly virulent.
[b] + = pycnidia produced; − = no formation of pycnidia.

celial cultures or gave mycelial sectors. A similar phenomenon was reported by Hansen and Snyder (5) for *Penicillium notatum* Westling.

In attempts to induce reversion of the mycelial type to the pycnidial type, cultures were made on a variety of natural and synthetic media; tubes were placed out of doors; and cultures on a variety of media were exposed to ultra-violet light for a range of time intervals. In no case was a reversion from a true mycelial type to a sporulating type observed.

In one experiment, seed was planted in sand infested 30 days previously with a pycnidial isolate. Severe infection of the seedlings resulted but pycnidia were very sparse. However, seedlings grown previously in the same sand had borne abundant pycnidia. In another case sand was infested with a mixture of equal parts of inoculum from a pycnidial and a mycelial strain. Pycnidial production on the seedlings grown in the infested sand was sparse although abundant pycnidia were produced on seedlings in sand infested with the pycnidial isolate only.

Twelve isolates in 13-months-old dehydrated cultures were all viable when transferred to fresh medium.

Morphology of the Pycnidium

The pycnidia produced by the sporulating isolates conformed to the description by Hansen of *Phoma terrestris* with the exception of one character.   In this investigation the pycnidia were found to be setose.   Hansen did not state that the pycnidium was smooth and he did not include a
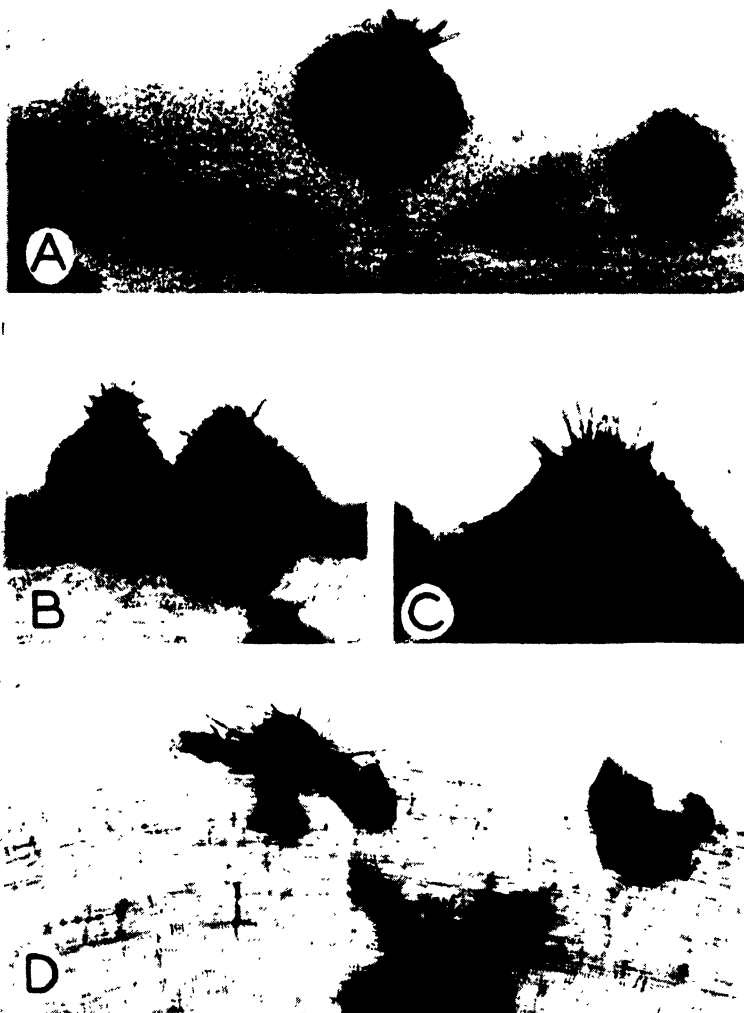
FIG. 1.   Pycnidia produced on onion roots.   × 99.   A. The California isolate on root of onion seedling grown in inoculated sand.   The pycnidia have short setae and are relatively deep-seated in the root tissue.   B and C. The Louisiana isolate on root of onion seedling grown in inoculated sand.   The pycnidia have long setae and are not so deep-seated as in the case of the California isolate.   D. The Louisiana isolate, after three single-spore transfers, on root from a bulb grown in infested soil.   The deformed and slightly smaller pycnidia are typical of those in pure culture after repeated single-spore transfers.

drawing or photomicrograph of a pycnidium. Carvajal (1) and Sprague (9) secured pycnidia in the cultures of some isolates but they did not describe or illustrate them.

The length, number and position of the setae on the pycnidium varied considerably among the different isolates. The setae of the California isolate (Fig. 1, A) were short, few in number, and grouped around the ostiole of the pycnidium. Those of the Louisiana isolate were longer and more numerous. They were scattered over the surface of the pycnidium but were most numerous about the ostiole (Fig. 1, B and C). The setae of the New York (Fig. 2, B), Illinois, Iowa, Wisconsin, and Colorado iso-
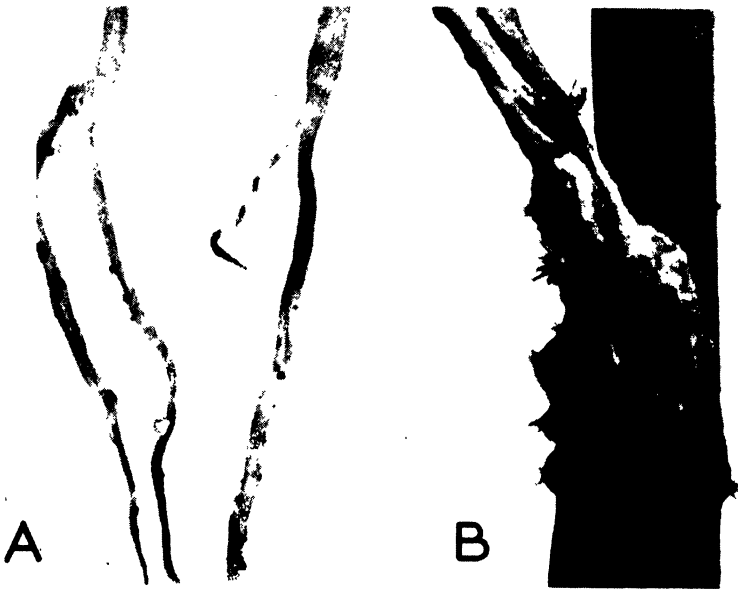
FIG. 2. Pycnidia produced on onion seedlings grown in sand culture. A. The Louisiana isolate on roots. ×5. B. The New York isolate on a dead cotyledon at the sand surface. ×49. The setae are concentrated around the ostiole.

lates were medium to long, few to numerous, and grouped almost exclusively about the ostiole.

The pycnidia also varied considerably in size, shape, and papillation. Those of the Louisiana isolate (Fig. 1, B) were typically globose and slightly beaked. Those of the other isolates tended to be subglobose and papillate, especially when produced on the host. An occasional pycnidium was found with two or even three ostioles. More variability was encountered in pycnidia produced in culture than in pycnidia produced on the host. After two successive single-spore transfers, the Louisiana isolate produced pycnidia which were less setose, more irregular in shape and smaller in size than those of the original isolate. The same type of pycnidium was produced in culture and on the host (Fig. 1, D).

Pycnidial Production on the Host

Pycnidia of the pink-root organism have not been reported previously on the host. This indicates that they are produced rarely on the plant in the field. Microscopical examination of diseased roots from the Racine, Wisconsin, area showed numerous pycnidial primordia varying in size but smaller than the mature pycnidium. Pycnidia were produced in cultures of isolates from these roots.

Mature pycnidia were first found in June, 1946, on the roots of onion seedlings grown in sand which had been infested with the first isolate secured from Louisiana specimens. This isolate also produced pycnidia in culture. Two successive single-spore transfers were made and mass transfers therefrom were used in all subsequent experiments with that isolate. A short time later pycnidia were found on seedlings grown in sand mixed with the roots and soil of the original consignment of specimens from Louisiana. Pycnidia were produced consistently on the host in sand-culture experiments in which this isolate was used as inoculum (Fig. 2, A). Moreover, pycnidia were produced on the roots in all cases in subsequent experiments in which the hydrogen-ion concentration of the host nutrient, salt concentration of the host nutrient, sand temperature, and host variety were varied.

The New York isolate produced numerous pycnidia on the dead cotyledons (Fig. 2, B) at the surface of the substrate in the sand-temperature studies. They were produced at all temperatures except 16° C. where the cotyledons of the seedlings were still alive at the end of the experiment. In another series pycnidia were produced on the dead cotyledons and on dead seedlings at the sand surface. Certain Texas, Iowa, and California isolates produced pycnidia in sand culture on dead plants above and below the sand surface, each in at least six different instances. Pycnidia were produced in sand culture on the dead outer scales of White Portugal bulbs inoculated with the Louisiana and California isolates. With soil culture, pycnidia were produced on the roots only by the Louisiana isolate (Fig. 1, D.).

Histological examination of diseased roots revealed the presence of pycnidial primordia in the cortical region of the root. Immature pycnidia were also found that were completely covered by the epidermis of the root or cotyledon. Serial sections of mature pycnidia on the roots revealed that in sections through the side of .the pycnidium the latter was completely covered by the host epidermis while in sections through the central portion of the pycnidium the latter protruded through the host epidermis (Fig. 3).

Taxonomy of the Fungus

The history of *Phoma* as given by Shear (7) reveals the taxonomic confusion that exists with this genus. It was set up by Fries in 1819 and was completely emended by Desmazieres in 1849. Saccardo's emended descrip-

tion of the genus in 1882 was as follows: perithecia smooth, without a beak, subcutaneous, not maculicolous, and spores without appendages. The characteristics of the genus as given by Grove (2, p. 58) are as follows:

> "PHOMA. Pycnidia covered by the epidermis or the periderm, then erumpent, membranaceous or subcoriaceous, occasionally carbonaceous, globose or depressed, glabrous, without a distinct or prolonged beak, but with a small (sometimes indistinct) ostiole which may be simply impressed, but is more often papillate. Spores, oblong or ovoid, more rarely fusoid, or subcylindrical, seldom globose, continuous, hyaline, typically biguttulate; sporophores simple, sometimes very short or imperceptible."
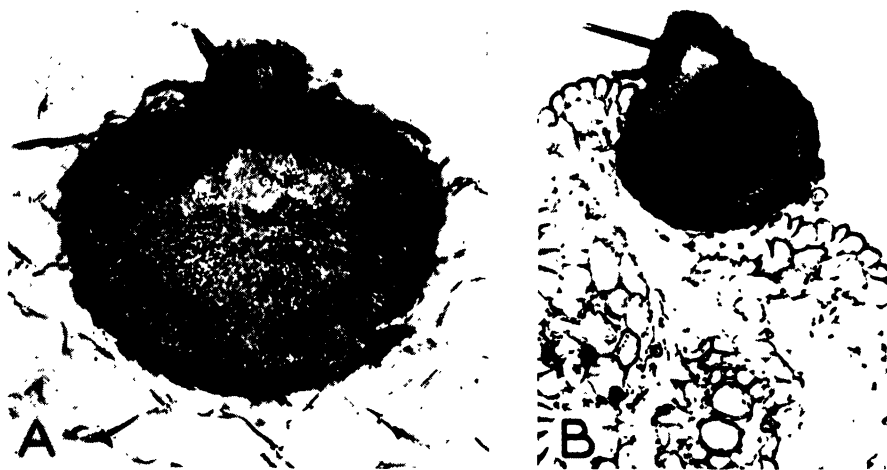
FIG. 3.   Cross-sections of pycnidia and host tissue from onion seedlings grown in inoculated sand.   A. California isolate on cotyledon.   × 260.   B. Louisiana isolate on root.   × 180.

The genus *Pyrenochaeta* was set up by de Notaris about 1845. His original description was not available but the one given by Grove (2) follows:

> "PYRENOCHAETA.   Pycnidia globose or conical, immersed, then erumpent, membranaceous or subcarbonaceous, pierced at the apex, beset with stiff bristles, especially toward the top; texture parenchymatous or prosenchymatous. Spores 1-celled, of various shapes, hyaline or faintly coloured; sporophores slender."
>
> "The characteristic of this genus lies in the possession of bristles clothing the external surface of the pycnidium; these are not so thick and stiff as those of Vermicularia."

The genus *Phoma* can no longer apply to the causal organism of pink root of onion because the genus is characterized as having a smooth pycnidium. In view of the fact that in each of the isolates in which pycnidia have been observed the latter have been setose, the species is trans-

ferred to the genus *Pyrenochaeta* and is designated as **Pyrenochaeta terrestris** (Hansen) nov. comb.

Morphology.—Pycnidia globose to subglobose, immersed, then erumpent, ostiolate, papillate to slightly beaked, dark brown to black, subcarbonaceous, 120–450 μ, slightly to definitely setose when mature, may occur singly, frequently gregarious. Setae light to dark brown, 1–5 septate, 8–120 μ in length, few to numerous, generally around the ostiole but may occur over the entire pycnidium. Conidia continuous, hyaline, oblong-ovoid, 3.7–5.8 × 1.8–2.4 μ, biguttulate, sessile in the pycnidium, escaping as a gelatinous mass through ruptures or as a cirrus through the ostiole. Mycelium septate, hyaline, guttulate.

Specimens are deposited at the following: Herbarium of the University of Wisconsin, Madison, Wisconsin; Herbarium of the Division of Mycology and Plant Disease Survey, Plant Industry Station, Beltsville, Maryland; Farlow Herbarium, Harvard University, Cambridge, Massachusetts.

DISCUSSION

Differences in morphology of the pycnidia of the various isolates of the pink-root organisms were very pronounced, especially between the California and the Louisiana isolates. Since all isolates caused the same disease symptoms it was considered advisable that all the strains be included under *Pyrenochaeta terrestris*. The emended description was made broad enough to include all the strains studied. It is interesting to speculate as to why the setae have not been noticed by other investigators. It should be noted that in the California isolate the setose characteristic was the least marked. It may be that Hansen's (3) original description was made on the basis of a similar isolate. Another factor was that the pycnidia produced in culture, especially on a medium high in dextrose, were overgrown with mycelium and the setose character was easily missed, especially if the characteristic was not marked. It was when pycnidia were found produced on the host that the setae attracted attention.

The pycnidia have never been found on the host in the field. Experiments with the Louisiana pycnidial isolate showed that pycnidia were produced on roots in unsterilized, artificially infested soil. However, none of the other isolates produced pycnidia on the host plant in soil even though some of them did so on seedlings in sand culture when the same inoculum was used. Inoculation experiments in sand and in soil with pycnidial and mycelial strains have shown that a pycnidial isolate must be used as inoculum before pycnidia are produced on the host. Other investigators have probably inoculated with mycelial isolates, or with inoculum from 20- to 45-day cultures of pycnidial strains which tend to become mycelial in character with age. In this investigation 10-day inoculum was used in all experiments. Many of the original isolations were mycelial in type. If they were pycnidal in type, special efforts, such as spore transfers or fre-

quent mycelial transfers, had to be made to keep them from becoming mycelial. Kreutzer (6) made extensive inoculation experiments, but only one of his isolates produced pycnidia in culture and thus only one pycnidial line was available to him.

A mycelial isolate was never found to revert to the pycnidial type and all attempts at artificial induction of pycnidia failed. The change from the pycnidial type to the mycelial type occurred very frequently when mycelial transfers were made from old pycnidial cultures. However, if transfers of young cultures were made continuously the line could be kept sporulating, probably indefinitely, by the use of mycelial transfers. The use of a mycelial transfer in itself did not cause a change from the pycnidial to the mycelial type. Pycnidial cultures tended to become overgrown with the mycelial type after they were 20 days old. This indicated a connection between aging of the culture and mutation to the mycelial type, a phenomenon noted in other fungi.

SUMMARY

A large number of isolates of the pink-root organism from various sections of the United States were assembled and their morphology was studied. In some of the isolates, pycnidia were produced in culture media and with every such isolate they were produced either on roots or cotyledons of plants grown in inoculated, white silica sand. In the case of only one isolate were pycnidia produced on the host grown in soil. This is the first report of pycnidia on the host, but as yet they have not been found in nature.

The pycnidia were found to be setose in all cases, although there was considerable variation between isolates in number and distribution of setae and in other morphological characteristics of the pycnidium. Because of the setose character of the pycnidium the species was transferred to the genus *Pyrenochaeta*.

Isolates which readily produced pycnidia in culture lost this character when transfers were made with mycelium from old cultures, in which there was a tendency for the fungus to mutate to the mycelial type. When sporulating cultures were transferred with mycelium from the advancing edge of the colony, or with spores, the sporulating character was retained. No production of sporulating mutants in mycelial lines was observed.

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APHID TRANSMISSION OF LILY VIRUSES DURING STORAGE OF THE BULBS

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Virus diseases constitute a major hazard in commercial cultivation of lilies. In the culture of Easter lily (*Lilium longiflorum* Thunb.) necrotic fleck and rosette diseases[3] must be avoided, preferably by selecting planting stock that is free from these diseases and planting it at a distance from known sources. Some garden lilies, notably *L. formosanum* Stapf and *L. tigrinum* Ker-Gawl., must also be kept free from the mottle virus[4] that is common in commercial stocks of Easter lily and Madonna lily (*L. candidum* L.) but is not obviously damaging in these species. Some growers therefore produce *L. formosanum* and other seed-propagated species in separate fields from the self-sterile and hybrid forms which must be propagated from bulbs. The usual practice, however, is to assemble all lily-bulb stocks in one storehouse for cleaning, grading, and packaging. Vector species of aphids may transmit viruses from sprout to sprout during this interval in the storehouse, and may thus vitiate the benefit from isolation provided by culture of the lily stocks in different fields.

Our attention was drawn to this problem by an Oregon grower who had produced virus-free *Lilium formosanum* under suitable isolation for several years, but later found an outbreak of lily mottle virus in the planting. No source of this virus could be detected near the field, but it was reported that the *L. formosanum* bulbs had been cleaned and graded in the same storehouse with *L. candidum* bulbs that had been grown elsewhere.

Experiments were therefore planned to determine whether *Aphis gossypii* Glover and *Myzus persicae* Sulz. would colonize on lily bulbs and sprouts during storage, and whether *A. gossypii* could transmit from sprout to sprout the persistent lily rosette virus, and *M. persicae* could transmit the nonpersistent lily mottle and cucumber mosaic viruses.

Lily mottle virus was taken from naturally infected stock of *Lilium longiflorum* var. Creole originally from Florida. The virus from this source material is similar to and possibly identical with that common in *L. candidum*. Cucumber mosaic virus, a strain originally isolated from necrotic-flecked stock of *L. longiflorum* var. Harrisi from Bermuda, was maintained in seedling Easter lilies. Lily rosette virus, originally obtained from Florida Easter lilies, was also cultured in Easter lily seedlings. Seedlings of

[3] Brierley, Philip, and Floyd F. Smith. Studies on lily virus diseases: the necrotic-fleck complex in *Lilium longiflorum*. Phytopath. **34**: 529–555. 1944.

[4] Brierley, Philip, and Floyd F. Smith. Studies on lily virus diseases: the mottle group. Phytopath. **34**: 718–746. 1944.

L. formosanum or *L. longiflorum* served as test plants for lily mottle virus and for lily rosette virus. As test plants for cucumber mosaic a stock of Creole lilies was selected that was free from necrotic fleck but which responded with fleck symptoms when infected with cucumber mosaic.[3] Potted plants in good vegetative growth or bulbs out of cool, dark storage with colorless shoot or sprout growth $\frac{1}{2}$ to 2 inches long were used to supply the virus and as test plants.

TABLE 1.—*Transmission Trials with Lily Mottle Virus, Cucumber Mosaic Virus, and Lily Rosette Virus by Aphis gossypii, Myzus circumflexus, and M. persicae*

Aphid species	Source of virus	Plants inoculated	Ratio of plants infected to plants exposed
		Lily Mottle Virus	
Myzus circumflexus	Sprouts of *Lilium longiflorum* var. Creole	Sprouts of seedling *L. longiflorum*	0/5
Myzus persicae	Do	Do	3/18
		Growing plants of *L. longiflorum*	2/5
		Growing plants of *L. formosanum*	10/10
		Bulb scales of *L. formosanum*	0/9
	Bulb scales of *L. longiflorum* var. Creole	Growing plants of *L. formosanum*	1/10
		Cucumber Mosaic Virus	
Myzus persicae	Growing plant of *L. longiflorum* seedling	Sprouts of *L. longiflorum* var. Creole	2/2
		Growing plants of *L. longiflorum* var. Creole	4/5
		Lily Rosette Virus	
Aphis gossypii	Sprouts of *L. longiflorum* seedling	Growing plants of *L. longiflorum*	3/5
		Sprouts of *L. longiflorum*	7/7
		Sprouts of *L. longiflorum* var. Creole	2/3

Myzus persicae, known from previous studies to be an efficient vector of lily mottle virus and of cucumber mosaic virus, was used in tests with these viruses. This species was cultured on cabbage (*Brassica oleracea* var. *capitata* L.), which is nonsusceptible to these viruses, and the aphids were starved for 2 hours before they were permitted to feed on source plants—a procedure known to improve efficiency of transfer of nonpersistent viruses. *M. persicae* fed readily on the leaf scales of sprouts of source bulbs after such a period of starvation. The aphids were placed on the sprouts of test bulbs and remained there for 2 days, but when moved to the green leaves of growing lilies all but a few individuals wandered off.

Myzus circumflexus Buckt., used in one test with lily mottle virus, was reared on potato (*Solanum tuberosum* L.) and starved for 3 hours before being placed on sprouts of the source bulbs. This species fed readily on the sprouts of stored lily bulbs. *Aphis gossypii*, reared on chrysanthemum (*Chrysanthcmum hortorum* Bailey), fed readily and increased to dense colonies on sprouts of stored Easter lily bulbs that served as sources of lily rosette virus. *A. gossypii* was permitted to feed for 6 days on this source material to allow it to acquire this persistent virus, before it was moved to sprouts or to growing plants of test lilies.

Mechanical inoculations from sprouted bulbs of Creole lilies affected with lily mottle virus were made on growing plants of *Lilium formosanum*. The Creole bulbs had been held in dry peat for $5\frac{1}{2}$ months at 50° F., and showed white shoot growth $\frac{1}{2}$ to 1 inch long but no root growth. Bulb scales were flaccid after long storage. The white shoot tips of 7 of these bulbs were ground in a mortar with a little water, and the macerated tissue was rubbed over 10 young *L. formosanum* plants. Three of these plants showed typical lily mottle symptoms. Symptoms were noted 10 days after exposure and confirmed after 30 days. A transfer of *Myzus persicae* (Table 1) from comparable material yielded 10 infections in 10 plants.

In a second test bulb scales of the same bulbs served as source of the virus. The outer, shriveled scales were discarded, and intermediate scales that were less flaccid were chosen. When ground with a little water they produced 8 infections in 10 inoculated plants, the symptoms and time interval corresponding to those in the first test. Another transfer of *Myzus persicae* from comparable material yielded 1 infection in 10 inoculated plants. These results show that the lily mottle virus is present in both scales and shoots of stored Easter lilies. They also imply that dormant lily bulbs might be indexed to determine the presence of this virus, but as this point has not been studied further, the efficiency of such indexing remains unknown.

The data in table 1 show that *Myzus persicae* can acquire the lily mottle virus from the colorless sprouts and the bulb scales of stored Easter lily bulbs. This aphid can transmit the virus to similar sprouts of Easter lily seedlings and to growing plants of this species or of *Lilium formosanum*. In a single trial, however, the aphid failed to introduce this virus into the bulb scales of *L. formosanum*—an indication that bulbs that have not begun to sprout in storage are less likely to become infected. *M. circumflexus* did not transmit lily mottle virus from sprout to sprout in storage. This species has also failed consistently to transmit this virus from plant to plant.[5]

Cucumber mosaic virus was readily transmitted by *Myzus persicae* from growing plants of Easter lily to growing plants of Creole lilies, or to the sprouts of these lilies in storage. Typical symptoms of necrotic fleck were induced in the Creole lilies in both tests.

Lily rosette virus was readily transmitted from sprout to sprout or from sprout to growing plants of Easter lilies by *Aphis gossypii*. It appears that

[5] See footnote 4.

this species can transmit this virus during storage, and can carry it to growing plants in nearby fields.   As *A. gossypii* colonizes readily on the shoots of stored bulbs, it also seems likely that this insect may be transported on them.   In addition to carrying lily rosette virus, this species is a known carrier of lily mottle, lily symptomless, and cucumber mosaic viruses.[6]

It is apparent that lily viruses of both persistent and nonpersistent classes may be transmitted during storage of the bulbs by appropriate aphid vectors.   Evidence is shown (Table 1) that one lily virus can be carried from growing plants to sprouting bulbs, and that two others can be carried from sprouting bulbs to growing plants.   It seems likely, therefore, that lily viruses can be carried from the field to the storehouse and from the storehouse to the field when suitable aphid species are present.   The hazard of long-distance spread by the flight of aphids is of course greater with the persistent viruses.   In planning segregation of tolerant and intolerant lily species, it is advisable to give consideration to the relation of the grading and storage facilities to the field plantings, and to keep aphids out of the storage house.

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[6] See footnotes 3 and 4.

THE INEFFICACY OF ETHYLENE CHLOROBROMIDE AS A THERAPEUTIC AGENT IN THE TREATMENT OF GARDENIAS INFECTED WITH THE ROOT-KNOT NEMATODE[1]

A. C. TARJAN

Tests were conducted at Beltsville, Maryland, in which 30 two-year-old cuttings of *Gardenia jasminoides* Ellis with roots badly infected with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, were subjected to various concentrations of ethylene chlorobromide.[2] The plants were divided into two groups, the first receiving only one application of the chemical, and the second receiving two applications of the chemical two weeks apart. The second application on the second group was planned for the following reason:—if the chemical was lethal to all living nematodes but failed to destroy the eggs, a two-week period after the first application would allow for the hatching of most of the eggs and the resulting nematode larvae would then be vulnerable to the toxic effects of the second application.

Plants which had been growing in six-inch pots were transplanted to eight-inch pots so that there would be a one-inch space, around the perimeter of the pots, which contained no roots. They were then watered and left for two days in order that moisture would be uniform throughout the soil. Since the maximum amount of chemical the plants would withstand had been roughly determined, in a previous experiment, to be below 0.50 ml., treatments applied were 0.40 ml., 0.25 ml., 0.10 ml., and 0.05 ml. of ethylene chlorobromide. Because of the small amounts of the chemical involved, distilled water emulsions were prepared so that each plant received a total of 8 ml. of the emulsion. Before application, the emulsion was thoroughly agitated in order that each replicate would receive the same percentage of ethylene chlorobromide in proportion to distilled water.

At the time of application, soil moisture was determined to be 7.01 per cent by weight while the soil temperature was 67° F. Method of application was as follows: four mutually equidistant holes were pierced two inches deep into the soil around the perimeter within each of the eight-inch pots containing the plants. Accuracy of dosage was attained by use of a graduated hypodermic syringe which injected four equal aliquots of the total amount into the holes in the soil of each pot; the holes were then sealed with soil. In this manner, none of the emulsion was poured directly on the roots since the perimeter of the root ball was located one inch from the perimeter of the pot. After treatment, all pots were watered lightly.

The second group received an additional treatment of 0.05 ml., 0.10 ml., and 0.25 ml. of the chemical in emulsion form after two weeks had elapsed; there was no additional application of the 0.40-ml. treatment since one application proved phytotoxic. Each group was harvested one month after its final treatment.

Preliminary inspections of a few galls after treatment revealed the presence of active nematodes. In an effort to obtain some type of tabular data that would illustrate the degree of efficacy of ethylene chlorobromide, quantitative determinations of parasite population in the roots were obtained by a modification of the Baermann technique—a method proven valid for obtaining root nematode population counts in studies of the meadow nematode. This method essentially consists of thoroughly washing the soil away from the roots of each plant, drying the root surfaces with filter paper, and then cutting them up finely until 5 gm. of root clippings are obtained.

TABLE 1.—*The effect of ethylene chlorobromide on root-knot nematode populations in Gardenia jasminoides roots*

Treatment	Root population counts						
	One application				Two applications		
	Replicates			Mean	Replicates			Mean
Control	30,	68,	79[a]	59	40,	45,	69	51
0.05 ml.	46,	80,	195	107	36,	41,	68	48
0.10 ml.	77,	109,	272	153	70,	108,	365	181
0.25 ml.	25,	52,	—[b]	39	—,	—,	—	—
0.40 ml.	—,	—,	—	—	—,	—,	—	—

[a] Each number represents the mean of nematode counts obtained from three 5-ml. samples.
[b] Plants died as a result of treatment.

This is put in the Baermann apparatus[3] which consists of a six-inch glass funnel in which a square piece of finely meshed cheesecloth is suspended by means of clamps. A short piece of rubber tubing attached to the end of the funnel is closed by means of a metal clamp. The root sample is put into the pocket formed by the suspended cheesecloth, and water is poured in until the root clippings are completely submerged. This apparatus is effective in that the nematodes evacuating the root fall to the cheesecloth, wriggle through the meshes, and fall to the neck of the funnel. In adopting this method for material infested with root-knot nematode, it should be pointed out that only the larval forms are capable of leaving the roots, consequently this method is in reality "an assay of the potency of the infection"[4] represented by the diseased root samples.

After allowing the root samples to remain in the funnels for 1½ days, a ten-ml. sample was drawn off from each funnel. This was increased in

[3] Filipjev, I. N., and J. H. Shuurmans Stekhoven, Jr. A Manual of Agricultural Helminthology. 878 pp. Leiden, Holland: E. J. Brill. 1941.
[4] As suggested by Dr. G. Steiner, Chief of the Division of Nematology, Bureau of Plant Industry, U.S.D.A., under whose supervision this work was carried out.

volume to 100 ml. by the addition of water, and, after thorough agitation, the nematodes in each of the three aliquots of five ml. were counted. The mean of these counts was then recorded.

With hopes of getting some sort of correlation between population counts and root condition previous to sampling, each root system was rated from "1" to "10," "1" signifying healthy roots and "10" signifying dead roots. No correlation, however, was apparent in the final analysis.

As shown in table 1, one application of 0.25 ml. and two applications of 0.05 ml. resulted in means lower than the controls. Although the results of the former treatment are explainable, no sound reason can be offered explaining why the 0.05 ml. treatment mean is less than the 0.10 ml. treatment mean within the two-application group. Another enigma is apparent in the lower mean of the controls in contrast to the 0.05 ml. and 0.10 ml. treatment means of the one application group and to the 0.10 ml. treatment mean in the two application group.

The explanation of these results may be: (1) The sampling method, as used in this experiment, may not have been reliable; (2) The fumigant may have weakened the resistance of the plant, thus increasing its susceptibility to penetration and subsequent infection by the root-knot nematode; (3) The fumigant may have killed the natural enemies (predatory nematodes and parasitic fungi) of the nematodes involved; or (4) There may have been a stimulating effect on the development of the parasites by the chemical treatments. Statistical analysis proved the results were not significant because of the wide variation of population counts within replicates.

The only figure which might illustrate a nematocidal tendency on the part of the soil fumigant were the results obtained using a treatment of 0.25 ml. in the one-application group. Since this treatment killed one of the replicates, its deleterious effects are not compensated for by the reduction of root nematode populations. Therefore, within the limits of this experiment, ethylene chlorobromide is not a suitable therapeutic agent for root-knot infested Gardenias.

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SIMULATION OF LILY ROSETTE SYMPTOMS BY FEEDING INJURY OF THE FOXGLOVE APHID

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Lily rosette, or yellow flat,[3] is one of the most destructive virus diseases of Easter lily (*Lilium longiflorum* Thunb.) and is also transmissible to other species of *Lilium*.[4] This is one of the most difficult diseases of lily to diagnose, for the symptoms of other diseases and injuries may resemble more or less closely the rosetting and curling effects of the rosette disease. We therefore consider that the only positive means of identifying this disease at present is the melon aphid (*Aphis gossypii* Glover), specific vector of the rosette virus. By this means this disease has been identified from field plantings in Florida twice and in Texas once. The rosette disease has not been confirmed from other field samples submitted. It is certain that similar diseases have been reported as rosette more than once. The most common symptoms that bear a strong resemblance to rosette result from cold injury, waterlogging, and diseases of the bulbs and roots, such as fusarium rot.[5] A less common but very similar symptom induced by the feeding of the foxglove aphid (*Myzus convolvuli* (Kltb.)) is described below.

Myzus convolvuli was collected on Easter lilies in a Long Island greenhouse in November, 1939, and again in 1945. Some curling of the infested leaves suggestive of rosette disease was present at both times. Aphids from the second collection were placed on an Easter lily seedling infected with rosette virus from November 26 to December 5, 1945, and then colonized on five healthy Easter lilies for 6 days. After removal of the aphids the plants were observed for 3 months, but no symptoms of rosette disease developed. This aphid is therefore eliminated as a possible vector of the lily rosette virus.

On December 11, 1945, twenty specimens of *Myzus convolvuli* were transferred to three additional healthy Easter lilies and allowed to feed and reproduce until January 18, 1946, when they were removed or destroyed. At this time all three plants showed marked downward curling of the young leaves on which the aphids had been feeding, and also an irregular yellow mottling of the old leaves (Fig. 1, A). These feeding effects had a very close resemblance to the symptoms of rosette disease (Fig. 2), and also suggested the presence of lily mottle disease. However, further growth of these

[3] Ogilvie, L. A. A transmissible virus disease of the Easter lily. Ann. Appl. Biol. 15: 540–562. 1928.

[4] Brierley, Philip, and Floyd F. Smith. Additional species of *Lilium* susceptible to lily rosette virus. Phytopath. 35: 129–131. 1945.

[5] Brierley, Philip, Floyd F. Smith, and F. P. McWhorter. Diseases of Easter lilies important in domestic production. Florists' Rev. 93 (2411): 21–23, 94. 1944.

FIG. 1.   Easter lily showing effects of feeding by foxglove aphids: A, Stunting, curling, and mottling in young growth; B, Persisting old injury and normal new growth in same plant five weeks after removal of aphids.

plants after removal of the aphids was normal (Fig. 1, *B*), the curling persisting at the zone of injury only.

Gadd and Loos[6] described a disease condition in *Lilium longiflorum* in Ceylon and also the symptoms of leaf curling and chlorotic spotting that developed after healthy lilies were colonized with green aphids of an unidentified species.   They also reported the development of chlorotic spots
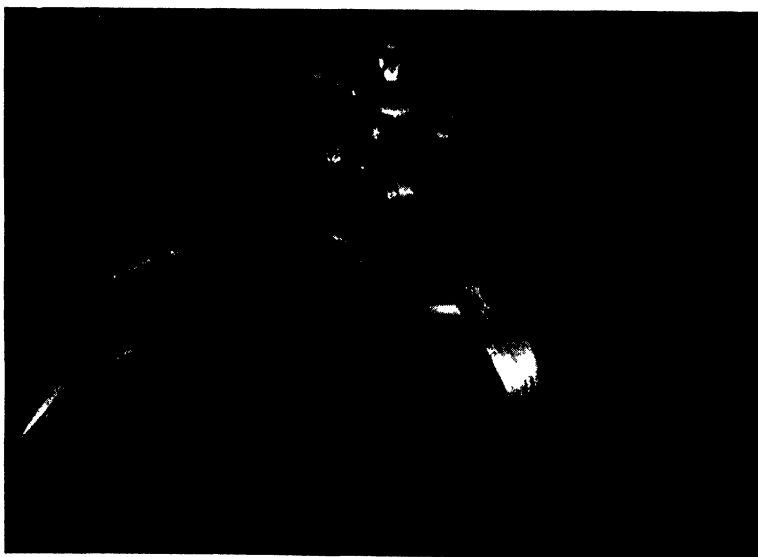
FIG. 2.   Easter lily infected with rosette disease by viruliferous *Aphis gossypii*.

[6] Gadd, C. H., and C. A. Loos.   Lily mosaic.   Trop. Agr. (Ceylon) 94: 160–167. 1940.

on cucumber, tomato, and turnip in about 5 days after the transfer of the same aphid from lily. They concluded that the virus involved was that of cucumber mosaic, although the symptoms were confined to the leaves fed upon by the aphids. The symptoms they illustrated were not typical of mosaic patterns and resembled the feeding injury by the foxglove aphid in our experiments. In response to our request Mr. Gadd sent us specimens of his green aphid, and P. W. Mason confirmed our identification of these specimens as being typical *Myzus convolvuli*. Mr. Gadd also indicated that the diseases of Easter lily in Ceylon were apparently a complex of viruses which included both necrotic fleck and rosette.

In the authors' experiments with lily viruses extending over several years, *Myzus convolvuli* failed to transmit lily mottle (0/29),[7] cucumber mosaic (0/8), lily symptomless virus (0/37), and lily rosette virus (0/8). Chlorotic spots or leaf curling, or both, followed in 5 or 7 days after this aphid was colonized on several of its many hosts, including African violet (*Saintpaulia ionantha* Wendl.), geranium (*Pelargonium hortorum* Bailey), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), turnip (*Brassica rapa* L.), and lily. The symptomatic period of the lily viruses was 10 days or longer when transmitted by their known aphid vectors.

It is clear that *Myzus convolvuli* is not a vector of either lily rosette virus or lily mottle virus, although its feeding injuries simulate the symptoms of both virus diseases.

This species is encountered only occasionally on lilies in greenhouses, but establishes persistent colonies where it occurs. Fairly large numbers on rapidly expanding young growth are n.eded to induce the injury simulating lily rosette, but it seems likely that such injury will be observed occasionally and may be misinterpreted by observers who are not forewarned.

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[7] Ratio of plants infected to plants exposed.

NUCLEAR INCLUSIONS PRODUCED BY A STRAIN OF TOBACCO MOSAIC VIRUS[1]

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INTRODUCTION

Although nuclear inclusions have been described in about 30 different virus diseases of animals, they have been reported much less frequently in plants. Hoggan mentioned finding square or oblong plates of undetermined nature in nuclei of solanaceous plants infected with tobacco mosaic virus (4). Kassanis (6) and Bawden and Kassanis (2) have reported protein crystals in the nuclei of solanaceous plants infected with severe etch or mild etch virus. McWhorter (8, 9) observed crystalline inclusions in both nuclei and nucleoli of leguminous plants infected with either Pisum virus 2 or Phaseolus virus 2. So far as we are aware, these constitute the only plant virus diseases in which nuclear inclusions have been described. These authors reported that the nuclear inclusions associated with the above mentioned legume and etch viruses were structurally rather stable, could be micromanipulated from the nuclei, and were not soluble under physiological conditions. Such inclusions do not occur in the nuclei of healthy plants. Although the inclusions induced by etch virus were very similar to the nucleoli in their staining reaction, Sheffield (10) has been able to demonstrate differences. She points out that in general these plant nuclear inclusions, which show protein color reactions, have many properties in common with the nuclear protein inclusions characteristic of the polyhedral diseases of *Lepidoptera*.

The present paper reports nuclear inclusions of types that so far as we can find have not been described before, although Hoggan (4) may have observed one of these types.

MATERIALS AND METHODS

Virus. The strain of tobacco mosaic virus (*Marmor tabaci* Holmes) which induces the formation of nuclear inclusions (1C) has been described elsewhere (5). For purposes of comparison, a strain of ordinary "green mosaic" (2A) and a "yellow mosaic" mutant (2A–1) were used.

Plant Material. Test plants included *Nicotiana tabacum* L. var. Turkish, *N. tabacum* L. var. Maryland Medium Broadleaf, *N. paniculata* L., *N. glutinosa* L., *Capsicum annum* L. var. California Wonder, and *Lycopersicon esculentum* Mill. var. Rutgers. Leaf tissue for histological study was prepared by infiltrating the intercellular spaces with a complete nu-

trient salt solution containing 5 per cent sucrose. The tissue was immersed in this solution in a flask, and suction applied intermittently. Then sections 4–5 cells thick were cut with a very sharp razor. Tissues so prepared may be kept alive for several days on the microscope slide if the solution is changed to provide oxygen.

SYMPTOMS

The "nuclear strain," 1C, induces a diffuse yellow spotting on inoculated leaves of *Nicotiana tabacum,* followed by a bright yellow systemic mottling similar to aucuba mosaic. Similar symptoms are produced on *N. paniculata, Lycopersicon esculentum,* and *Capsicum annum.* Localizing necrotic lesions are produced on *N. glutinosa.* Nuclear inclusions have been observed in all of these species, except *N. glutinosa,* when infected with virus 1C.

PROPERTIES OF THE VIRUS

With respect to thermal inactivation and resistance to desiccation, virus 1C behaved in a manner typical of tobacco mosaic virus. Material dried one year or heated to 80° C. for ten minutes induced typical symptoms and nuclear inclusions.

Physical Properties. With respect to size and shape, the particles of all three strains are typical for tobacco mosaic (5). Virus 1C, however, has a somewhat higher isoelectric point and can be separated from mixtures with 2A by cataphoresis (5).

INTRACELLULAR INCLUSIONS

Three types of inclusion bodies have been observed in affected cells. (a) Crystalline or fibrous proteinaceous inclusions in the cytoplasm, (b) loosely aggregated amorphous proteinaceous precipitates in the cytoplasm, the "X-body" type, and (c) inclusions in the nucleus which are apparently identical or very similar to the types formed in the cytoplasm. Inclusions have not been found in nucleoli.

The crystalline or fibrous inclusions are very unstable and go into solution when the cell is mechanically injured. The amorphous precipitates are not destroyed by such treatment. The unstable type of inclusions in both cytoplasm and nucleus present a wide variety of forms (Fig. 1 and 2), although within lesions of the same age on the same leaf considerable uniformity exists.

The number of both nuclear and cytoplasmic inclusions reaches a maximum in from 10 to 20 days after infection of the cells. Beyond this time these inclusions have been observed less frequently. Similar behavior of cytoplasmic inclusions in ordinary tobacco mosaic has been described (7).

Nuclear inclusions of the types described here were never observed in non-infected tissues. It is noteworthy that in spite of the polymorphism of the inclusions there was a marked tendency for them to take the same form in the nucleus and cytoplasm of the same cell (Fig. 1). This would

indicate that the conditions affecting the orientation of particles as the inclusions are formed are similar in the cytoplasm and nucleus of a cell. An exception was the rarely observed ring-shaped inclusion (Fig. 2, E). This type was found in the nuclei only. They strongly resemble the rings and figures of eight, described by Suchkov (11) in the cytoplasm of mosaic-infected gramineae.

The cytoplasmic inclusions induced by strain 1C, and those formed by typical green and yellow mosaic (2A and 2A-1) seem to have many properties in common. On treatment with HCl at pH 1.3 they all break down, frequently with formation of needles. If treated with saturated ammonium sulfate they do not break down when the cell is ruptured or treated with certain reagents. Such salt treatment makes possible various tests.
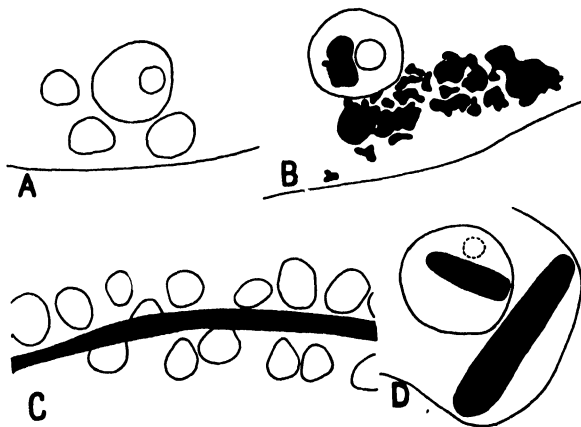
FIG. 1. Nuclear and cytoplasmic inclusions in living leaf cells of *Nicotinia tabacum* L. var. Turkish infected with tobacco mosaic virus strain 1C. In all drawings viroplastic inclusions appear as solid black. A. Nucleus and plastids of non-infected cell. B. Cell with amorphous trypan blue-positive nuclear and cytoplasmic inclusions. C. Portion of long fibrous cytoplasmic inclusion lying among plastids. D. Elongated platelike nuclear and cytoplasmic inclusions in one cell. All figures represent line drawings over original photomicrographs. 785×. A striate structure not shown in the drawings was visible in the fibrous inclusions.

The inclusions then give a red coloration with Millon's reagent, and an orange yellow color when treated with concentrated HNO_3 and NH_4OH. These inclusions are therefore probably proteinaceous. Trypan blue (0.05 per cent) intensely stains the non-crystalline inclusions ("X-bodies") induced by all three virus strains whereas the crystalline or fibrous inclusions do not so stain. The nuclear inclusions induced by virus 1C behave in all these respects like the cytoplasmic inclusions.

Killed Nuclei. Nuclei of cells infected with virus 1C and killed in sectioning, often presented a very characteristic appearance not observed in cells infected with the other viruses. In many instances all of the inclusion material had gone into solution leaving a very large clear space (usually occupying most of the nuclear volume) with the visible structural material of the nucleus, including the nucleolus, surrounding it. The nucleoli were

FIG. 2.   Photomicrographs of living Turkish tobacco mesophyll cells containing nuclear and cytoplasmic inclusions.   A. Fibrous inclusions.   B. Platelike inclu.ion surrounded by clear space in nucleus.   C. Platelike inclusion in cytoplasm.   D. Block-like nuclear inclusion.   E. Ring-shaped inclu.ion surrounding nucleolus.   All pictures 1535×.   A line drawing of A appears in figure 1.

apparently not changed structurally. Treatment of such nuclei with saturated ammonium sulfate generally resulted in precipitation in the central clear space of masses of closely packed parallel needles. These needles gave positive Millon's and xanthoproteic reactions. Some damaged nuclei (not treated with ammonium sulfate) contained within the central clear space extensive needle- or spike-like crystalline deposits, usually much larger than those formed following treatment with ammonium sulfate. Such forms were not observed in undamaged nuclei.

SUMMARY AND CONCLUSIONS

All of the evidence to date shows that the nuclear inclusions induced by tobacco mosaic virus strain 1C are similar or identical to those occurring in the cytoplasm of the same cells. The nuclear and cytoplasmic inclusions induced by this strain reacted in all our tests like the various proteinaceous cytoplasmic inclusions found in cells infected with ordinary types. As Beale (3) and Bawden (1) have pointed out, the crystalline inclusions found in these infections are probably composed at least in part of virus. The present data suggest, therefore, that a strain of tobacco mosaic virus exists which is capable of invading nuclei of cells.

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BLOSSOM AND SPUR BLIGHT (SCLEROTINIA LAXA)
OF SOUR CHERRY

E . C . C A L A V A N A N D G . W . K E I T T [1]

Blossom and spur blight of sour cherry (*Prunus cerasus* L.), incited by *Sclerotinia laxa* Ader. and Ruhl., was discovered in Baileys Harbor Township of Door County, Wisconsin in 1941 (21). Subsequently, the fungus was found in the Sturgeon Bay cherry district, where several local outbreaks occurred. Although its natural occurrence in the eastern half of North America was established only recently (21), *Sclerotinia laxa* has been a serious pest for many years in most other humid stone-fruit regions of the world, with the exception of Australia and New Zealand (14, 16, 35, 42, 44).

The time and manner of introduction of *Sclerotinia laxa* into North America are unknown. The first detailed cultural studies of American isolates were made by Posey (31, 32) in 1913 in Oregon. At that time spur blight was already established in most humid stone-fruit areas in British Columbia and the Pacific Coast States and probably had been present in central California since about 1900 (6, 16). In the Pacific Coast States *S. laxa* has become the major brown-rot organism in some localities (3, 16, 23). East of the Rocky Mountains it has been reported only from Wisconsin.

Sclerotinia laxa has been studied extensively in Europe and western North America, but no comprehensive work concerning its development and satisfactory control on sour cherry under conditions closely approximating those of Wisconsin has been reported. This paper presents the results of experimental studies from 1941 to 1945 concerning this pathogen and its control on sour cherry in Wisconsin. A preliminary report has been published (9).

SYMPTOMS

The first symptoms on sour cherry appear as brown lesions on the petals or as a browning of the stigma, style, or stamens. In stigma infection the darkened area spreads down the style (Fig. 1, A) to the ovary, from which it extends outward to other flower parts and downward through the pedicel. From a diseased pedicel, infection may extend to the supporting twig or branch. With stamen infection, browning usually appears at the tip of the filament but soon progresses to the rim of the floral cup, where a small, light-brown lesion is formed at the juncture of the stamen and the rim (Fig. 1, B). This spot enlarges into a dark-brown, "U"-shaped area on the side of the floral cup (Fig. 1, C). From the floral cup the browning extends through the receptacle and pedicel. Occasionally infection may reach
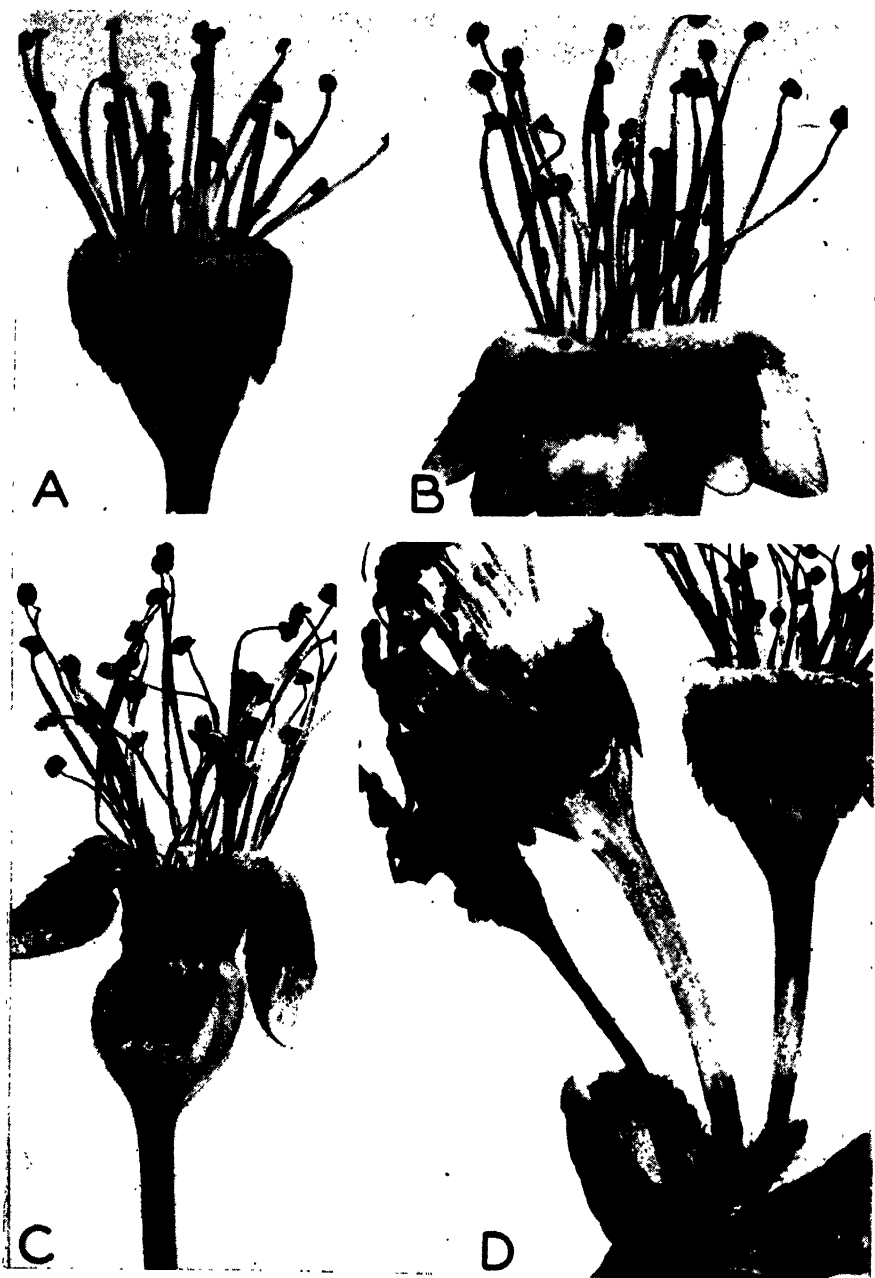
FIG. 1. Sour cherry blossoms infected by *Sclerotinia laxa*. A. Lesion on the style following stigma infection. B. Small lesion on the floral cup following stamen infection. C. Advanced lesion on the floral cup. D. Diseased blossom cluster showing spread of infection from the base of one pedicel into two others.

the rim of the floral cup from a diseased petal and produce symptoms resembling those resulting from stamen infection.

The fungus spreads readily from the base of a pedicel to surrounding bracts and to other pedicels of the cluster, and thus it rots the pedicels of flowers which previously may have escaped infection (Fig. 1, D).

Wilting of blossom clusters occurs within 4 to 15 days after infection. During moist weather such clusters may bear a loose layer of gray, sporulating hyphae or occasional small sporodochia. Leaves on diseased spurs may wilt at any time from 10 days to 6 weeks after blossom infection. Pendant clusters of shriveled leaves and blossoms (Fig 2, E, F) constitute the most conspicuous manifestation of this disease.

Frequently the fungus grows from killed spurs into larger branches, where its invasion often leads to the formation of elliptical cankers (Fig. 2, G). When the attack is especially severe, branches may be girdled and the distal portions killed within a few weeks (Fig. 2, E). Gum pockets commonly are present near the margins of young cankers, and masses of clear to amber-colored gum often exude from diseased spurs and branches.

Gray sporodochial tufts (Fig. 2, A, B, C), usually less than 1.0 mm. high and from 0.5 to 3.0 mm. in diameter, form in the spring from shortly before budbreak (in Wisconsin) until shortly after bloom. Sporodochia may develop anywhere on the dead twigs or adherent parts but are especially abundant between bud scales, on the bracts, and in the floral cups of overwintered blossoms.

Although *Sclerotinia laxa* is most important as a blossom- and twig-blighting organism, it also is able to attack both green and ripe fruits, upon which it induces symptoms very similar to those of American brown rot incited by *Sclerotinia fructicola* (Wint.) Rhem. Decaying fruits darken and may be covered by numerous spore tufts during moist weather.

Leaf infection, except by direct contact of leaves with diseased material, is rare. However, it was obtained experimentally in the greenhouse following inoculation by conidial suspensions. Lesions appeared first as small, chlorotic spots which soon became gray to brown. Under very moist conditions the necrotic areas enlarged rapidly (Fig. 2, D) and occasionally extended down the petiole to the shoot, as reported on sweet cherry by Maier and Mittman-Maier (26).

The symptoms described above agree in many respects with those reported for European brown rot of stone fruits on various hosts in Europe and western North America (10, 18, 24, 44).

THE CAUSAL ORGANISM

The taxonomy, synonymy, and history of *Sclerotinia laxa* Ader. and Ruhl. and closely related species have been reviewed by many authors (7, 13, 15, 17, 27, 35, 42) since the first brief description of *Torula fructigena*, from specimens possibly containing both *S. laxa* and *S. fructigena*, was published by Persoon (30) in 1796. Some uncertainty remains, however, regarding the

FIG. 2. *Sclerotinia laxa* on sour cherry. A-C. Sporodochia on overwintered dead parts. D. Leaf lesions. E. Blighted terminal shoot. F. Blighted spurs. G. Branch canker.

ultimate disposition of certain questions concerning the correct binomial for the supposed *S. laxa* of North America. Among the phenomena which merit further investigation are the ability of an isolate of *S. fructicola* to assume the colony characters of *S. laxa* under certain conditions (Fig. 3), the existence of numerous variants with colony characters *in vitro* intermediate between those typical of *S. laxa* and *S. fructicola* (Fig. 4, G-J), and the apparent absence of apothecia of *S. laxa* in North America. Possibilities exist that North American isolates herein designated as *S. laxa* may be variants of another species (possibly *S. fructicola*), hybrids, heterocaryons, or a species of *Monilia* having no perfect stage.

Reference of the fungus inciting blossom and spur blight of sour cherry in Wisconsin to *Sclerotinia laxa* Ader. and Ruhl. is based on comparisons of
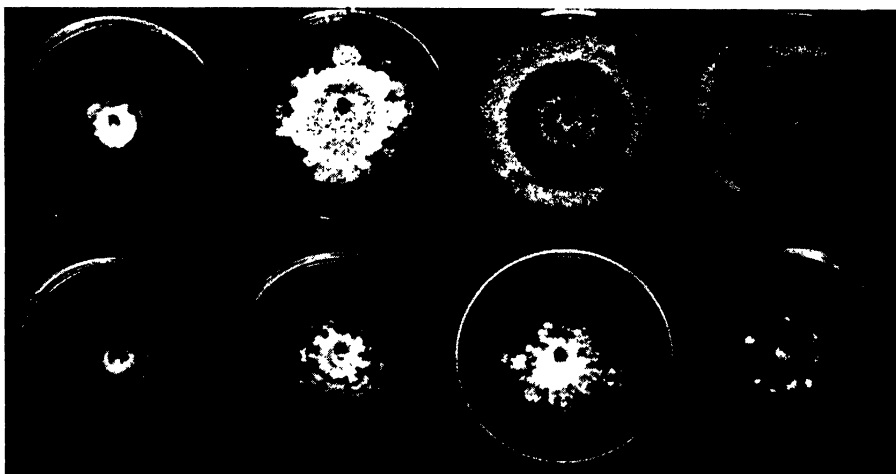
FIG. 3. Colonies of *Sclerotinia fructicola* (upper row) and *S. laxa* (lower row) on potato-dextrose agar 33 days at the following temperatures: left to right; 4°, 8°, 12°, and 16° C. Cultures in the upper row are from plum at Madison, Wisconsin; those in the lower row, from sour cherry at Baileys Harbor. Note lobing and zonation of the *S. fructicola* cultures at 4° and 8° C.

Wisconsin isolates with isolates of the organism widely accepted as *S. laxa* obtained from California, Oregon, and Washington and on numerous descriptions and illustrations of the fungus in American and European literature (4, 10, 13, 16, 28, 31, 41, 43). This identification of the fungus is regarded as tentative pending possible discovery of its perfect stage and the development of a better basis for differentiating *Sclerotinia* spp. on stone fruits.

Colony characters. On potato-dextrose agar and on many other artificial media, *Sclerotinia laxa* characteristically formed rounded lobes at the margin of the colony (Fig. 3). However, different isolates varied greatly in the number and width of lobes produced (Fig. 4), and some isolates from almond (California) formed only a few lobes.

Concentric zonation is another important colony character of all Wiscon-
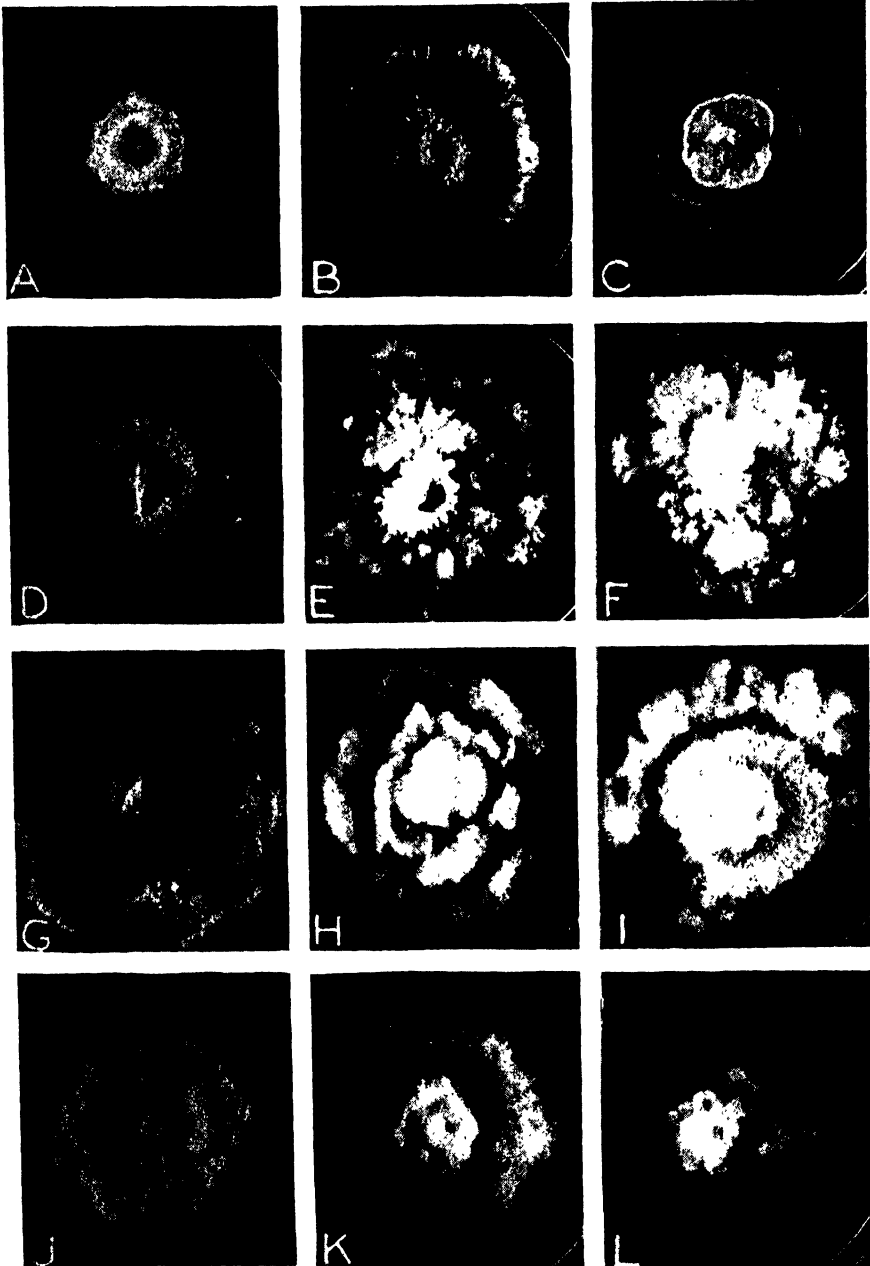
FIG. 4. Colony forms of *Sclerotinia laxa* on potato-dextrose agar 20 days at 16° C. A–C. Nos. 71, 67, and 93, from immature fruits of *Prunus cerasus*, Baileys Harbor, Wisconsin. D. No. 1, from spur of *P. cerasus*, Baileys Harbor. E. No. 86, from spur of *P. pennsylvanica*, Baileys Harbor. F. No. 51, from twig of *Chaenomeles japonica*, Corvallis, Oregon. G. No. 68, same source as A–C. H, I. Nos. 35 and 36, from blossoms of *P. Amygdalus*, California. J. No. 50, from mummified fruit of *P. domestica*, Corvallis, Oregon. K, L. Nos. 42 and 41, from twigs of *P. Amygdalus*, California.

sin isolates and most others examined. Zones apparently are produced by the periodic interruption of radial growth in favor of a thickening of the mycelial mat. After each interruption radial growth is resumed, usually from points other than the apices of the lobes, but the new growth remains very thin as long as radial growth continues. Lobing and zonation in artificial cultures of *Sclerotinia laxa* have been discussed extensively by Killian (24).

The color of cultures of *Sclerotinia laxa* on potato-dextrose agar or on other media may vary with different isolates. Young cultures are usually white or gray until the first interruption of their radial growth, which normally occurs when they are 20 to 40 mm. in diameter. At about this time the sub-surface mycelium begins to darken, particularly at the place of seeding and near the margins of the lobes. As the colony ages this mycelium often becomes black or greenish black, though in a few isolates it turns yellow or reddish brown. The aerial hyphae usually retain their light color longer than those in contact with the substratum.

Sporulation in culture. The scarcity of *Sclerotinia laxa* conidia in culture has been recorded by Berkhout (5), Ezekiel (13), and many others. It has constituted a serious problem to workers desiring to make large-scale inoculations by means of spore suspensions.

Most isolates of *Sclerotinia laxa* produced few or no conidia on potato-dextrose agar and various other artificial substrata at moderate temperatures. Therefore, various methods were tried to obtain sufficient conidia for inoculation experiments. Petri-dish cultures on nutrient, nutrient-dextrose, corn-meal, malt, oat, potato-dextrose, and prune agars, prepared according to the formulae in Riker and Riker (33), were made and incubated at 20° C., but very few spores formed. Cultures on steamed potato plugs gave only slightly better sporulation.

Many conidia were formed, however, in about 10 weeks on potato-dextrose agar cultures of various Wisconsin isolates grown at low temperatures (4° to 8° C.). Equally good results were obtained in about 8 weeks when cultures were started at 16° to 20° and transferred to 4° C. after 10 to 15 days. This treatment made possible the production of large amounts of conidial inoculum at any season, but required the maintenance of many cultures.

Conidia of *Sclerotinia laxa* were formed also on inoculated apples and pears. Wisconsin isolate 1, when inoculated into overripe Bosc pears and incubated in a moist chamber at 20° C., produced well-developed sporodochia within 60 hours. Under similar conditions small sporodochia were formed on Emperor grapes in 36 hours. On apples sporulation was slow and variable. Many sporodochia were produced on 3 of 5 inoculated Jonathan apples in 5 days at 20° C., but very few spores appeared on 5 Wealthy, 5 Red Delicious, and 10 McIntosh apples similarly treated. Good sporulation occurred on fresh sour cherries, but not on canned or frozen sour cherries, frozen peaches, or on dried prunes.

The best yield of conidia was obtained by growing the fungus on Emperor grapes. For best results the grapes were used raw or heated to 60° C. for 10 minutes. Relatively few conidia formed on autoclaved grapes. With the exception of a few isolates from California, abundant sporulation developed in 3 or 4 days in cultures on slightly heated grapes.

Microconidia, almost spherical and about 2.5 μ in diameter, were produced in tremendous numbers by most isolates of *Sclerotinia laxa* on various substrata under a variety of environmental conditions. The microconidia are formed in chains on tiny sterigmata borne by club-shaped structures. They are imbedded in a matrix and appear on the surface of a culture as slimy masses of variable color, usually cream, yellow, dark green, or black. The sexual function of microconidia of *S. Gladioli* (Massey) Drayton has been discussed by Drayton (12), but the sexual nature of microconidia of *S. laxa* has not been demonstrated.

Apothecia of *Sclerotinia laxa* have not been produced in culture on artificial media. Their occasional occurrence on mummified fruits has been reported from Europe (1) and the British Isles (40), but not from North America. Stalked structures that suggest apothecial fundaments, ¼ to ½ inch high, formed in some cultures of isolate 62 (Washington) and of isolate 21 (Wisconsin) but failed to mature into apothecia.

Temperature relationships. The effect of temperature on the radial growth rates of *Sclerotinia laxa* isolates 2, 21, and 23 was studied briefly, and comparative tests were made with *S. fructicola* isolates 4 and 12. Each culture was grown on 20 cc. of 2 per cent potato-dextrose agar in a Petri dish. The cultures were started by placing a standardized seeded disc, 3 mm. in diameter and 2 mm. thick, of 2.5 per cent dextrose agar at the center of each Petri dish. Triplicate cultures of each isolate were incubated at 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 37° C. Measurements of radial growth were made after 2, 4, 7, 14, and 30 days. Radial growth of the 3 *S. laxa* isolates and isolate 12 of *S. fructicola* in 4 days was greatest at 28°, whereas *S. fructicola* isolate 4 grew most rapidly at 24° C. In 7 days *S. fructicola* grew more than *S. laxa* at 12°, 16°, 20°, and 24°, but less at 4° C. At 8° C. *S. fructicola* isolate 4 grew faster and isolate 12 slower during the first week than any of the 3 *S. laxa* isolates, but in 2 weeks both *S. fructicola* isolates were far ahead of *S. laxa*. At 28° C. differences in growth rates between isolates of the same species were greater than between certain isolates of different species. At 32° C. *S. fructicola* grew more rapidly than *S. laxa*. No growth of either occurred at 37° C.

Conidial size. Conidia of numerous isolates of *Sclerotinia laxa* and *S. fructicola* had the same general characteristics when examined microscopically. They formed in branched chains without true disjunctors, but, when mature, they broke apart easily at the middle of wedge-like thickenings between the spores. As the spores separated from each other, they became roughly lemon-shaped, although there was considerable variation in relative dimensions, so that some were nearly spherical.

Measurements of conidia from various sources were made, and the average dimensions are recorded in table 1. Each solitary spore in fields chosen at random was measured in order to obtain valid average dimensions. Figures obtained compare favorably with those given by other workers (7, 13, 25, 27, 31, 34, 42). Considerable variation in spore size was apparent between samples produced at different temperatures or on different substrata. Spores produced on dead, overwintered plant parts were usually smaller than those formed on fresh fruit or on artificial media. Those formed at low temperatures tended to be smaller than those produced at 20° to 25° C. Similar phenomena have been noted by Hewitt and Leach (16), Killian (24), Ritzema Bos (34), and Wormald (42). It is apparent that conidial

TABLE 1.—*Measurements of conidia of Sclerotinia laxa and S. fructicola*

Species, isolate, and source	Substratum	Temperature	Average dimensions[a]
		°C.	*Microns*
S. laxa			
2 *Prunus cerasus* twig, Wis.	Steamed potato plug	22	13.9 × 18.5
2 Do	Emperor grape	22	10.8 × 16.1
21 Do	Potato-dextrose agar	20	10.9 × 14.8
23 Do	Sour cherry blossom		12.8 × 17.5
23 Do	Potato-dextrose agar	20	10.7 × 14.9
23 Do	Do	4	9.3 × 12.0
100 Do	Sour cherry twig		9.1 × 13.2
101 *Prunus cerasus* green fruit, Wis.	Green sour cherry fruit		11.7 × 14.4
87 *Prunus pennsylvanica* twig, Wis.	Emperor grape	22	9.3 × 13.7
50 *Prunus domestica* mummy, Ore.	Potato-dextrose agar	22	8.8 × 14.1
102 Do	Italian prune mummy	.	7.3 × 10.6
S. fructicola			
4 *Prunus domestica*, Wis.	Potato-dextrose agar	22	9.2 × 12.8

[a] Figures based on measurements of 100 spores per sample. See text.

measurements alone provide insufficient basis for differentiation between *Sclerotinia laxa* and *S. fructicola*.

Spore germination. Branched and crooked growth of the germ tubes of *Sclerotinia laxa* soon after conidial germination has been mentioned by Ezekiel (13), Roberts and Dunegan (35), and Wormald (44) as a characteristic of this species useful in distinguishing it from *S. fructicola*. Ezekiel germinated spores of each species in hanging drops of potato-dextrose decoction at 25° C., and after 18 hours found the germ tubes of *S. laxa* to be branched and crooked, whereas those of *S. fructicola* were typically unbranched and straight.

Experiments made in this laboratory reveal that characteristic germ tube differences between the two species may not occur under certain conditions. Conidia of *Sclerotinia laxa* isolate 23 and *S. fructicola* isolate 12 were germinated over deep-well slides in hanging drops of distilled water and in 0.1 and 0.01 per cent dextrose solutions. Very little branching occurred in any

drop, and the only difference noted was the slightly more tortuous nature of some *S. laxa* germ tubes. Characteristic differences in branching were noted, however, when conidia of the two species were made into spore suspensions over grape cultures upon which they had been grown. When drops of these spore suspensions, which contained considerable nutrient material from the grapes, were incubated at various temperatures from 12° to 32° C., the *S. laxa* conidia produced crooked, branched germ tubes quickly, while the germ tubes of *S. fructicola* usually remained straight for 24 hours or more. It is evident from these tests that growth-habit differences between the germ tubes of the two species are readily apparent only under certain nutritional conditions favorable to their expression.

Conidia of *Sclerotinia fructicola* germinated more quickly in distilled water or weak sugar solutions than conidia of *S. laxa. S. laxa* conidia, regardless of age, required at least 4 hours for germination in distilled water at 24° C., whereas conidia of *S. fructicola* only 2 days old germinated under the same conditions in 75 minutes. The addition of 0.1 per cent dextrose to distilled water reduced the minimum time for conidial germination of *S. laxa* to about 2 hours at 24° C.

The optimum temperature for *Sclerotinia fructicola* conidial germination was 24°, but for *S. laxa* 20° C. appeared slightly more favorable. Spores of both species germinated readily from 16° to 32° C. Some germination occurred at 4° and at 34° but not at 37° C.

DISEASE DEVELOPMENT STUDIES

Methods and materials. All blossom inoculations were made on trees 2- to 4-years-old set in 8-inch pots at least 14 months before inoculation and forced into bloom in the greenhouse about February 1. Blossoms were inoculated, except as otherwise stated, by means of a conidial suspension sprayed from an atomizer attached to a compressed-air line. All spore suspensions used in large-scale inoculations were mixed from 2 or 3 isolates. In the hope of obtaining an inoculum more representative of the species, mass-isolated rather than single-spore cultures were used for inoculation work.

Immediately after inoculation, trees were placed in a saturated atmosphere in large moist chambers. Two types of chamber were used. One type, which provided condensation water adequate for infection and disease development has been described by Keitt and Jones (20). The second type, of temporary construction, made use of mist to maintain high atmospheric moisture. A fine, relatively uniform mist was produced by means of atomizers connected to about 20 pounds air pressure and water reservoirs. To reduce temperature fluctuations, 1 or 2 thermostatically controlled, 250-watt, gooseneck heaters were installed in each mist-type moist chamber, except the one at 11° C. where no heater was necessary. Temperature variations were about plus or minus one degree centigrade except in the 11° house, which, though usually between 10° and 12°, occasionally reached 13° C.

Inoculated trees were kept in a moist chamber at least 3 days to permit the fungus to become well established in the blossoms. After this, in 1944, they were allowed to dry about 8 hours (4:00 p.m. to midnight) each day. It was thought, however, that a proportionately greater amount of time in a wet environment might favor more severe attacks of brown rot. Therefore, in 1945 the spray was turned off only about 8 hours (9:00 a.m. to 5:00 p.m.) every second day, and once each week the dry period was lengthened to 24 hours to permit thorough drying of the trees and wilting of diseased branches. The same treatment was accorded chambers at all temperatures.

TABLE 2.—*Relation of temperature to infection of sour cherry blossoms by Sclerotinia laxa*

Year and variety	Incubation temperature	Blossoms inoculated[a]	Blossoms infected[b]
	°C.	Number	Per cent
1944	Initial[c]		
Montmorency	10	272	14
	20	152	72
	30	218	94
	Secondary[d]		
Montmorency	11	126	40
	16	101	44
	20	163	52
	24	155	68
	28	97	69
	Continuous[e]		
1945	11	187	81
Montmorency	16	132	92
	20	142	97
	24	186	98
Early Richmond	11	52	94
	16	167	96
	20	219	94
	24	128	98

[a] Includes all open blossoms, with or without petals.
[b] Data taken 5 days after inoculation in 1944, and at 5, 6, 7, and 13 days at 11°, 16°, 20°, and 24° C., respectively, in 1945.
[c] Temperature during first 48 hours after inoculation.
[d] Temperature maintained following initial 48-hour incubation (see text).
[e] Temperature maintained from inoculation.

Preliminary Experiment in 1943. In March, 1943, a preliminary greenhouse experiment demonstrated the pathogenicity of Wisconsin isolate 1 of *Sclerotinia laxa* on blossoms of Montmorency and Bing cherries and on blossoms of Hale Haven peach.

Experiments in 1944. The main experiment in 1944 was confined to Montmorency blossoms because no Early Richmond blossoms were available. Blossoms were inoculated with mixed conidial suspensions of 3 *Sclerotinia laxa* isolates known to be virulent and given a 48-hour incubation period in a chamber which provided condensation water adequate for infection. Incubation temperatures used during this period were 10°, 20°, and 30° C.

After 48 hours the trees were removed to the mist-type moist chambers. Blossoms from each of the 3 groups (10°, 20°, 30° C.) were distributed as evenly as possible among the 5 chambers (at 11°, 16°, 20°, 24°, and 28° C.). Blossom infection data were taken 5 days after inoculation, but before the appearance of secondary infection.

The results, which are shown in table 2, are compiled separately according to the initial temperature (in the inoculation chamber) and the secondary or later incubation temperature. It is recognized, however, that in this experiment the effects of the initial and the later incubation temperatures cannot be sharply differentiated.
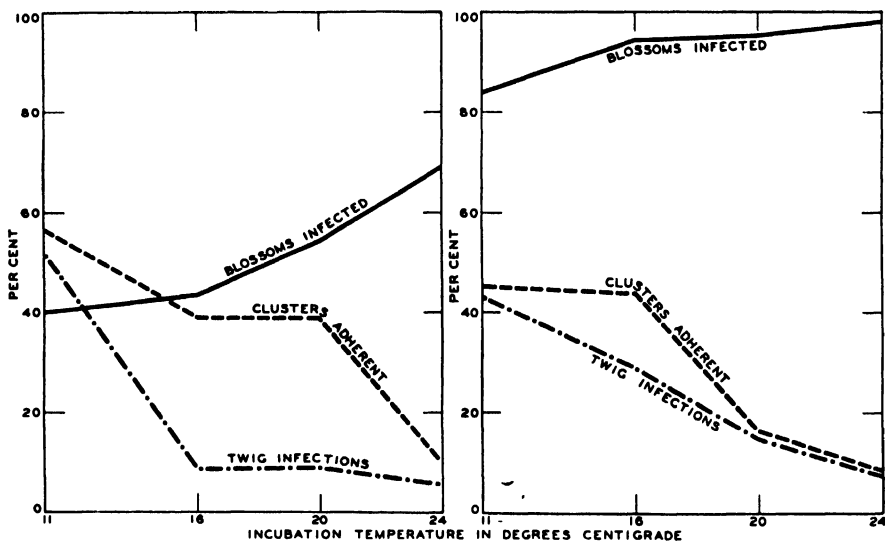
FIG. 5. Results of inoculations of young sour cherry trees with *Sclerotinia laxa* conidia, in the greenhouse, showing (a) percentages of primary blossom infection, (b) blossom clusters adherent to the twigs 6 weeks after inoculation, and (c) blossom clusters from which twigs became infected within 8 weeks following inoculation. Data were taken in 1944 (left) from Montmorency only, and in 1945 (right) from Montmorency and Early Richmond varieties.

Thirty degrees centigrade appeared to be the incubation temperature (first 48 hours) most favorable to blossom infection and 10° least favorable. Of the 5 temperatures used after the initial incubation period, 11° seemed to be least favorable and 24° and 28° C. most favorable.

Because only a relatively small number of blossoms was moved from each of the 3 inoculation groups to each of the 5 temporary moist chambers, no detailed table of results from these 15 subdivisions is presented. However, it was found that only 2 of 62 blossoms inoculated at 10° and kept at 11° C. were diseased in 5 days, whereas 97 of 99 blossoms inoculated at 30° and removed after 48 hours to 24° or 28° C. were infected.

It was observed that many infected blossom clusters were abscised prior to twig infection. Therefore, records were taken in all chambers to determine (a) the number of blossom clusters adherent to the trees 6 weeks

after inoculation and (b) the number of clusters from which the fungus had entered the wood or bark of the supporting twig within 8 weeks after inoculation. The results of this study are shown graphically in figure 5.

The critical period for blossom infection, on the basis of the studies in 1944, begins with the opening of the blossoms and continues until the floral cups turn yellow and wither (Table 3). At ordinary temperatures blossoms remain susceptible 1 to 2 weeks.

Although few yellowed blossoms were inoculated, and though such blooms were very susceptible to attack through withered and damaged stamens, it was observed that the infection spread so slowly in the floral cups and pedicels that it usually did not reach the base of the pedicels before the blossoms dropped.

Experiments in 1945. Both Early Richmond and Montmorency blossoms were available for inoculation in 1945, and improved facilities per-

TABLE 3.—*Incidence of primary infection by Sclerotinia laxa of sour cherry blossoms inoculated at different stages of development*

Year and variety	Floral development[a]	Blossoms inoculated	Blossoms infected[b]
		Number	*Per cent*
1944	Petals unopened	243	2
Montmorency	Petals opened	389	53
	Petals fallen	253	57
1945	Green-tipped bud	119	10
Early Richmond	White-tipped bud	355	30
	Petals opened	994	95
	Petals fallen	283	91

[a] At hour of inoculation.
[b] Data taken according to statement in text.

mitted the distribution of trees of each variety among the various incubation temperatures immediately after inoculation. The concentration of spores in the inoculum was doubled over that used in 1944. Spore suspensions were prepared from conidia produced on grape cultures.

Records were taken on each variety to determine (a) the number of blossoms showing primary infection at different temperatures, (b) the number of buds or blossoms infected after inoculation at different stages of development, (c) the part or parts of blossoms where infection occurred, (d) the number of blossom clusters adherent after 6 weeks, and (e) the number of blossom clusters from which the fungus entered the wood within 8 weeks (Fig. 5).

The numbers of blossoms infected at different temperatures are shown in table 2. In order to avoid including secondary infections, the counts were made 5, 6, 7, and 13 days after inoculation at 24°, 20°, 16°, and 11° C., respectively. Except at 11° C., there was little apparent difference in varietal susceptibility. High percentages of infection were obtained at all temperatures, particularly on Early Richmond, but with Montmorency

blossoms there appeared to be a slight trend to more infection at higher temperatures. The increase in amount of infection obtained in 1945, over that in 1944, is attributed chiefly to the increased spore load of the inoculum.

The critical period for infection, as in the 1944 experiment, began with the opening of the blossoms. Although some buds inoculated while still in the green-tip stage were infected, it was observed that no infection occurred until after the petals were exposed. Infection of white-tipped buds usually followed the opening of the flowers, but sometimes took place earlier through the exposed outer surfaces of the petals. The data on infection are based on the stages of blossom development at inoculation rather than at infection.

Results from both varieties have been combined in table 3. No important varietal differences were apparent in this experiment, although a slightly higher percentage of infection occurred with Early Richmond than with Montmorency. The site of blossom infection was almost invariably on an anther, stigma, or petal. In this connection anthers and stigmas appeared to be of greater importance than petals, which though often infected, usually dropped before the pathogen entered the rim of the floral cup. Primary infection of other flower parts was rarely seen. The frequency of infection through anthers and stigmas of 813 perfect flowers is shown in table 4.

Microscopic examinations of infected anthers consistently revealed germinated pollen and fungus hyphae, and sometimes fungus hyphae passing from the anthers into the pointed tips of the filaments. Almost pure cultures of *Sclerotinia laxa* were isolated repeatedly from diseased anthers.

A special experiment was conducted to determine by direct inoculation of selected floral parts those through which the fungus is most likely to enter the flower.

Inoculations were made by application either of dry conidia or droplets of conidial suspension to specific floral parts. Certain unwanted blossom structures were removed before inoculation. Stamens of blossoms receiving style inoculations were cut off, and the number of stamens was reduced to five on blossoms receiving filament or anther inoculations. Anthers were removed from all inoculated filaments. In this experiment all trees were incubated at 20° to 24° C. in a moist chamber which provided sufficient condensation moisture for infection, but not enough to permit the formation of large drops which might have displaced the inoculum.

Stigma inoculations gave the highest percentage of infections; anthers the next highest (Table 5). It was noted that unruptured anthers rarely were invaded, while uninjured petals were found to be somewhat susceptible. Styles were rarely attacked directly, and no direct infection of filaments was observed. However, 24 styles, each inoculated about 3 millimeters below the stigma, became infected later by way of the stigmas. Only 1 sepal, which had been injured, was infected.

TABLE 4.—*Incidence of primary infection through anthers and stigmas of Early Richmond and Montmorency sour cherry blossoms sprayed with a conidial suspension of Sclerotinia laxa, 1945*

Variety	Blossoms inoculated	Blossoms infected[a]			
		Via anthers only	Via stigma only	Via anthers and stigma	Total
	Number	Per cent	Per cent	Per cent	Per cent
Early Richmond	353	34.6	0.0	57.5	92.1
Montmorency .	460	41.3	0.7	48.9	90.9
Total ..	813	38.4	0.4	52.6	91.4

a Data taken soon after appearance of primary lesions.

Considerable leaf infection developed at 16°, 20°, and 24° C. The greatest amount occurred at 20°, but very little occurred at 11° C. It is possible that inoculum may have been driven into the leaf tissues by the force of the spray from the atomizer, for no leaf infection developed on 50 sound leaves inoculated with drops of spore suspension carried on a wire loop. Most leaf infection seen on orchard trees followed direct contact of the leaf with an infected blossom or blossom part, but leaf lesions similar to those developed in the greenhouse (Fig. 2, D) have been described and illustrated by Maier and Mittman-Maier (26) on sweet cherry.

The rate of disease development at 5°, 11°, 16°, 20°, 24°, and 28° C. was studied. These standard temperatures were maintained within 1° C., except in the 11° C. chamber where occasional variations of 2° C. occurred. The approximate incubation period required for the development of certain symptoms at each of the above temperatures was determined (Table 6). The disease progressed most rapidly in the blossoms at 24° and was but little slower at 20° C. At 5° C. no infection was visible for 6 days, and 9 more days were required for production of lesions on the rim of the floral cup. There was practically no difference between Early Richmond and Montmorency varieties in the rate of disease development on the blossoms.

TABLE 5.—*Incidence of infection by Sclerotinia laxa of various specially inoculated parts of Early Richmond and Montmorency sour cherry blossoms, 1945*

Floral part inoculated	Inoculum	Units inoculated	Units infected[a]
		Number	Per cent
Stigma	Dry conidia	13	92
Stigma	Conidial suspension	6	100
Anther	Dry conidia .	40	35
Style	Conidial suspension	50	10
Filament	do	78	0
Petal (outer surface)	do	15	0
Petal (inner surface)	do	15	20
Sepal ... .,	do	50	2[b]

a Final readings made 8 days after inoculation.
b This sepal showed evidence of mechanical injury.

Sclerotinia laxa apparently required a considerably longer incubation than *S. fructicola*. Weaver (37) reported infection of peach blossoms by *S. fructicola* only 5 hours after inoculation at 24°, and 18 hours after inoculation at 10° C.

<center>CONTROL</center>

Preliminary experiment in 1942. A preliminary experiment on the control of spur blight was made in an orchard which had been severely damaged by *Sclerotinia laxa* in 1941. Materials used were (a) Elgetol (sodium dinitro-ortho-cresolate), 0.5 per cent, (b) Fermate (ferric dimethyl dithiocarbamate), ¾–50, (c) copper-lime-monocalcium arsenite and cold-pressed menhaden fish oil, 3–2–2 (1½ pints fish oil) –50, and (d) Bordeaux mixture, 3–4–50. The selection of the formula for the copper-lime-arsenite mixture

TABLE 6.—*Approximate minimum incubation periods for appearance at stated temperatures of certain symptoms on Early Richmond and Montmorency sour cherry blossoms inoculated with conidia of Sclerotinia laxa*

Variety	Incubation temperature	Minimum period for expression of stated symptoms			
		Tips of styles or filaments browned	Lesions on calyx tube	Tip of pedicel browned	Entire pedicel[a] browned
	°C.	Hours	Hours	Hours	Hours
Early Richmond	5	144	360	432	504
	11	48	132	152	186
	16	36	62	90	160
	20	15	38	58	87
	24	14	·35	52	80
	28	20	˗ 44	70	108
Montmorency	5	144	360	432	504
	11	48	132	152	186
	16	36	62	90	175
	20	20	42	63	100
	24	16	35	52	80
	28	20	44	70	108

ᵃ Pedicel length approximately 25 mm.

was based on results obtained by Keitt and Palmiter (22). Spraying was unavoidably delayed until May 16, just as the trees were breaking into bloom.

The copper-lime-monocalcium arsenite mixture stopped sporulation almost completely and gave visibly better results than Elgetol. Bordeaux mixture and Fermate appeared to reduce the number of blighted spurs, but neither halted the formation of sporodochia.

The application of Elgetol or copper-lime-arsenite to the trees during bloom resulted, as expected, in considerable injury to the blossoms and young leaves, but the twigs were not severely damaged. The suppression of sporodochial production and the absence of serious injury to the trees by copper-lime-monocalcium arsenite indicated its probable value as an eradicant dormant spray for the control of *Sclerotinia laxa*.

Experiments in 1943. Control work was continued in 1943 on Early Richmond trees about 15 years old in an experimental area containing 5 blocks of 24 trees each and one of 6 trees. Tests were confined to 2 eradicant materials, applied just before budbreak, and Bordeaux mixture, 3–4–50, applied early in bloom.

An eradicant spray of copper-lime-monocalcium arsenite and fish oil, 3–2–2 (1½ pints fish oil) –50 was applied to blocks II and III. In the preparation of this material the copper sulphate and then the lime were put in the tank to form Bordeaux mixture. Next the monocalcium arsenite and finally the fish oil were added. The 6 trees in block I received monocalcium arsenite, 2–50, without Bordeaux mixture or fish oil. Blocks IV and V received no dormant spray. The eradicant dormant sprays were applied May 6, when a few buds were beginning to swell. Thorough applications, with considerable run-off, were made in an effort to bring the spray materials into direct contact with all diseased parts.

A protectant spray of Bordeaux mixture, 3–4–50, was applied June 1 to blocks III and IV, when about three-fourths of the blossoms were open. All experimental spraying was done from the ground. Block V was used as an unsprayed check.

Data were taken on sporodochial suppression and on incidence of spur blight from 6 representative trees in each of the 5 blocks. On June 8, at the end of the critical period for infection, 10 overwintered, diseased spurs were collected at random from each of these trees. Subsequently, counts of sporodochia were made on 30 spurs taken at random from each sample. These counts included sporodochia on or within adherent flowers, fruits, leaves, bracts, and bud scales.

The blossom spray alone had little or no effect on the suppression of sporodochia, but the eradicant dormant sprays proved very effective. Monocalcium arsenite, 2–50, on the basis of these counts, reduced the production of sporodochia 83 per cent; but the copper-lime-monocalcium arsenite and fish-oil mixture was significantly more effective, with 94 per cent sporodochial suppression (Table 7).

Counts of blighted spurs were obtained July 2 by examination of a total of 300 spurs, not over 3 inches in length, on each of 6 trees per block (Table 8). Spurs counted were on branches taken at random around the perimeter of each tree.

The incidence of spur blight was greatly reduced in all sprayed blocks. The use of copper-lime-monocalcium arsenite and fish-oil preparation followed by the bloom spray (block III) gave the most striking results, 99 per cent reduction in incidence of spur blight. However, an analysis of variance of figures for spurs blighted in blocks II to V, inclusive, showed no significant differences between results for blocks II and III. Control either in block II or block III was significantly better than in block IV at odds of 19 to 1. Block I was not comparable, so could not be included

TABLE 7.—*Effectiveness of eradicant dormant sprays in limiting the sporodochial production of Sclerotinia laxa on overwintered dead spurs of Early Richmond sour cherry trees, 1943 and 1944*

Block and treatment[a]	Spurs examined[b]		Average sporodochia per spur		Indicated reduction in sporodochia by spray	
	1943	1944	1943[c]	1944[d]	1943[c]	1944[d]
	Number	Number	Number	Number	Per cent	Per cent
I Monocalcium arsenite ..	30	....	7.5	... ...	83	.
II Copper-lime-monocalcium arsenite + fish oil	30	60	2.8	4.1	94	85
VI Copper-lime-monocalcium arsenite + dormant spray oil		60	.	0.7	....	97
V None	30	60	43.1	26.2	...	....

a Dates of application: May 6, 1943; April 30, 1944. See text for dosages.

b Dates of spur collection: June 8, 1943; June 22, 1944.

cData based on counts of total sporodochia on 30 spurs from each treatment. See text.

d Data based on counts of total sporodochia on 60 spurs (10 from each of 6 trees) per treatment.

in the analysis of variance. Counts on 2 trees in block I showed approximately 7 per cent of the spurs blighted.

Slight injury developed on some trees receiving the dormant spray alone. Twig and branch injury was confined to weak or wounded trees.

Experiments in 1944. These experiments were made on Early Richmond trees about 15 years old in an orchard approximately ⅓ mile from the 1943 plots. The experimental area was divided into 5 blocks of 20 trees each, plus a sixth block containing only 12 trees.

Two eradicant spray preparations were tested in 1944. Copper-lime-monocalcium arsenite and fish oil 3–2–2 (1½ pint fish oil) –50, was applied to all trees in blocks II and III. Copper-lime-monocalcium arsenite and Stanolind dormant spray oil, 3–2–2 (1½ pints fish oil) –50, was used on the 12 trees in block VI. This mixture was included in the experiment in the hope

TABLE 8.—*Effectiveness of early sprays in the control of brown-rot spur blight on Early Richmond sour cherry trees, 1943*

Block and treatment[a]	Spurs examined[b]	Spurs blighted	Indicated reduction by spray
	Number	Number	Per cent	Per cent
II *Dormant,* copper-lime-monocalcium arsenite + fish oil	1800	23	1.3	97.5
III *Dormant,* copper-lime-monocalcium arsenite + fish oil; *Bloom,* Bordeaux mixture	1800	9	0.5	99.1
IV *Bloom,* Bordeaux mixture	1800	170	9.4	82.1
V None	1800	948	52.7	....

a Dates of application: Dormant, May 6; Bloom, June 1. See text for dosages.

b Data taken on 300 spurs per tree. See text.

of finding a suitable oil to replace the fish oil component in the dormant spray. Monocalcium arsenite, 2-50 (block I), because of its inferior record of sporodochial suppression in 1943 was not included in the 1944 program. The dormant sprays were applied April 30 when the buds were just beginning to swell.

Bordeaux mixture, 3-4-50, was the only protectant bloom spray used. It was applied to the trees in blocks III and IV on May 28, when only about one-fourth of the blossoms were open, and was used as a late-bloom spray in block VII on June 2, after about two-thirds of the petals had fallen.

Data were taken on sporodochial suppression, incidence of blossom blight, and incidence of spur blight. Counts of sporodochia were made only in blocks II, V, and VI. Ten overwintered, blighted spurs were col-

TABLE 9.—*Effectiveness of early sprays in the control of brown-rot blossom blight on Early Richmond sour cherry trees, 1944*

Block and treatment[a]	Blossoms examined[b]	Blossoms infected	
	Number	*Number*[c]	*Per cent*
II *Dormant,* copper-lime-monocalcium arsenite + fish oil	1800	17	0.9
VI *Dormant,* copper-lime-monocalcium arsenite + dormant spray oil	1800	8	0.4
III *Dormant,* copper-lime-monocalcium arsenite + fish oil; *Early bloom,* Bordeaux mixture	1800	20	1.1
IV *Early bloom,* Bordeaux mixture	1800	27	1.5
VII *Late bloom,* Bordeaux mixture	1800	83	4.6
V None	1800	79	4.4

[a] Dates of applications: Dormant, April 30; Early bloom, May 28; Late bloom, June 2. See text for dosages.
[b] An equal number of blossoms was examined from 6 trees per block.
[c] Minimum significant difference at odds of 19 to 1 = 28.7.

lected at random from each of 6 trees per block and examined to determine the numbers of sporodochia present (Table 7).

Either dormant spray preparation provided good suppression of sporodochia, although a few sporodochia were present on some spurs when the sprays were applied; but the mixture containing dormant spray oil was significantly more effective at odds of 19 to 1 than that made with fish oil. Very little injury was apparent from either treatment.

Blossom blight data were taken in every block. On June 4, four days after the beginning of the first infection period, 300 blossoms from clusters selected at random were examined on each of 6 trees per block. Despite the brief infection period substantially fewer blighted blossoms were found in all sprayed blocks, except block VII, which was sprayed after the infection period, than in unsprayed block V (Table 9). Differences among blocks II, III, IV, and VI were slight, as might be expected in mild attacks.

There was little blossom blight or spur blight in 1944 because dry weather prevailed during the critical period. Only 2.8 per cent blighted spurs were

found in the unsprayed block, and the most severely diseased unsprayed tree had only 9 per cent blighted blossoms. In order to obtain any indication of the measure of control by different treatments, it was necessary to determine the total number of blighted twigs (spurs and terminals) on each of the trees selected from each block. On the basis of these figures the dormant spray with a supplementary bloom spray, as in 1943, gave the best control (92 per cent). Bordeaux mixture during early bloom apparently reduced spur blight about 60 per cent, whereas the dormant spray alone reduced it only 50 per cent. No spur blight was found in block VI where the copper-lime-monocalcium arsenite plus dormant spray oil was used. The late bloom spray in block VII gave no reduction of blossom or spur blight.

<div align="center">DISCUSSION</div>

The existence of biotypes intermediate between typical isolates of *Sclerotinia laxa* and *S. fructicola* is apparent (Fig. 4). Ezekiel (13) illustrates several cultural types of each species. Hewitt and Leach (16) show a culture of *S. fructicola* which, though it has the usual concentric rings and considerable sporulation, has also an irregular, somewhat lobed margin like *S. laxa*. On the other hand, many lines of *S. laxa* isolated by us from specimens obtained from California and Oregon formed many aerial hyphae but sporulated less readily than *S. fructicola*.

Wisconsin isolate 4 obtained from a plum mummy near Madison, where no *Sclerotinia laxa* has been found, appeared in culture as a typical colony of *S. fructicola* at 12° to 32°, but at 4° and 8° C. it formed lobes and zones closely resembling *S. laxa* (Fig. 3). However, isolates from the margins of such lobes reverted to the typical *S. fructicola* growth habit when incubated at higher temperatures. Thus, at least with this isolate, the growth habit of *S. fructicola* appeared almost identical with that of *S. laxa* at 4° or 8° C. No instance of a similar change of growth habit was observed in *S. laxa*.

The stability in culture of various isolates of *Sclerotinia laxa* and *S. fructicola* suggests the possible genetic nature of certain variants. Anastomoses between different lines may possibly result in new and intermediate types. Intraspecific anastomoses are common, and Ezekiel (13) observed an interspecific anastomosis between *S. laxa* and *S. fructicola*. Moreover, natural mating of *S. laxa* and *S. fructicola* in areas where both species occur is a possibility not seriously discounted by the fact that no apothecia of *S. laxa* have been reported in North America. Experimental interspecific and intraspecific matings of these fungi may eventually do much to clarify this matter.

The results of the present work indicate that moderate to high temperatures (16° to 30°) are more favorable to blossom blight than temperatures of 11° C. or lower, and that infection and disease development on blossoms are most rapid at about 24° C. However, it should be noted, that although

the progress of the disease was retarded by low temperatures, it was possible to obtain a high percentage of infected blossoms at 11° C., particularly with a concentrated spore suspension such as that used in 1945.

Temperatures while the fungus is invading the blossoms probably are no more important than those prevailing during subsequent dry periods. The occurrence of warm weather after the blossoms have been destroyed but before the fungus has entered the wood of the supporting twigs favors rapid drying of the diseased parts and the maturation of abscission layers at the bases of the pedicels and, thereby, the dropping of the diseased flowers. Even when the environment is almost continuously moist and the temperature relatively constant, as in the incubation chambers, the results indicate that blossom clusters are, eventually, more subject to abscission at high or moderate than at low temperatures (Fig. 5).

The effect of different temperatures on the spur-blight phase of brown rot, because of the small number of spurs blighted in the greenhouse, was less striking than for blossom blight. However, at 11° C. there was less abscission of diseased blossoms and, consequently, more spur blight than at higher temperatures.

A preliminary experiment with *Sclerotinia laxa* blossom blight under conditions of controlled humidity gave results agreeing closely with those obtained by Weaver (37) for *S. fructicola* blossom blight on peach and cherry. Weaver found that at relative humidities above 90 per cent the diseased areas remained fairly moist, whereas at 80 per cent the lesions dried out and were delimited. It is apparent that while the blossom-blight phase is greatly favored by constant conditions of abundant moisture and moderate temperature, the spur-blight phase of European brown rot probably develops more readily under a complex of fluctuating moisture and temperature conditions.

The respective lengths of moist and dry periods necessary for extensive development of spur blight were not investigated, but it was observed that under conditions prevailing in the incubation chambers the fungus usually grew no farther than the short axis within the bud scales. It was observed that very little additional moist weather was necessary for the development of spur blight after the fungus had rotted the pedicels.

Many statements concerning the court of blossom infection for European brown rot of stone fruits are found in the literature. Woronin (45) considered the stigma to be the only normal infection court. This opinion was accepted by most pathologists until Wormald (39) in 1919 obtained infection of plum flowers by placing spores at the bottom of the floral cups, on the "discs" and on the stigmas. Chabrolin (10) in 1924 reported that under natural conditions infection of apricot blossoms arises through the calyx, particularly through the tube, and through the filaments of the stamens as well as through the stigma. He believed that, because of their much smaller area, the stigmas were less frequently infected than the

floral envelope, but he lacked sufficient evidence to prove it. He found hyphae present in the anthers between the pollen grains, but apparently he did not consider the anthers to be infection courts. Maier (25) in 1942 recognized that infection may occur through either the stigma or the receptacle. Weaver (37) found that *Sclerotinia fructicola* was capable of infecting all floral organs of peaches; but no reference to anthers as important infection courts for *S. laxa* has been found. However, according to the experiments reported herein, blossom infection originates in anthers more often than in any other floral part; but the progress of the fungus as it travels downward through one or several filaments, usually somewhat obscured by about 30 other stamens, is not easily observed. The true nature of most "floral-cup" or "receptacle" infections was revealed only by a series of careful observations beginning not more than 14 hours after inoculation. Under close scrutiny nearly all such infections have been found to originate in the anthers. Although experiments indicated that a lower percentage of inoculated anthers than stigmas was attacked (Table 5), the greater number of anthers (25 to 35) per flower rendered blossom infection through one or more anthers more probable than stigma infection. Even though 10 per cent of 50 styles experimentally inoculated became infected, these infections were slow in appearing, and had the anthers or stigmas of these flowers been inoculated at the same time as the styles, the styles would have rotted from above or below before infection occurred through their own surfaces. Most blossom infections, by natural or by artificial inoculations, originated in an anther or a stigma, where the host tissues were not resistant to attack and a plentiful food supply was available to the developing hyphae.

Early Richmond trees were used in the spraying experiments because they had been observed in Wisconsin, and reported by Huber and Baur (19) in Washington, to be much more severely attacked than Montmorency by *Sclerotinia laxa*. Treatments were applied to blocks of trees rather than to single-tree plots to reduce the influence of air-borne conidia from near-by trees receiving different treatments.

The selection of spray materials and schedules tested in 1943 was based upon a study of the usual control practices for *Sclerotinia laxa* in Europe and North America and upon consideration of the promising results of experiments with eradicant fungicides in Wisconsin (22) and California (38). Recommended control measures for *S. laxa* in England (11, 29) include (a) the destruction of the diseased plant parts, (b) a dormant spray of 10 per cent tar oil before the buds begin to swell, and (c) a spray of Bordeaux mixture (about 3–4½–50) just before the blossoms open. Anres and Joessel (2) advised sprays of Bordeaux mixture, 2 per cent, on apricots in France when the buds are opening and at the beginning of blossoming. In Germany, Bucksteeg (8) suggested 4 sprays of unspecified materials to be applied (a) when the buds begin to swell, (b) just before

the blossoms open, (c) just after petal fall, and (d) 2 or 3 weeks after petal fall. Rudolph (36) in 1925 reported that in California insecticide oil sprays applied to apricots during the winter frequently showed fungicidal properties. For blossom blight control he advised a dormant spray of crude-oil emulsion, 30–200, a dormant spray of Bordeaux mixture, 8–8–50, and 1 or 2 applications of Bordeaux mixture of various strengths during bloom.

Keitt and Palmiter (22) in 1937 reported the results of tests made over a 13-year period with various eradicant fungicides. They found that suitable copper-lime-arsenite mixtures were highly effective against fungal fruiting structures at the surface or near a permeable surface. Wilson (38) reported in 1942 the results of experiments with arsenite sprays to control *Sclerotinia laxa* on almonds, apricots, plums, and prunes in California. He found monocalcium arsenite, 2 or 4 pounds per 100 gallons, to be an effective eradicant spray.

Less emphasis is placed on the results of the control experiments in 1944 than in 1943 because of the sparse development of lesions in 1944. In 1943 there was an almost continuous 5-day infection period early in bloom, with considerable wind and rain. Following this the weather was almost ideal for spur blight development. In 1944, however, although abundant inoculum was present because early rains had favored sporulation, the blossom period was so dry that little infection occurred. There was only one light shower (about 0.2 inch) during bloom.

The proper time for application of the dormant spray in Door County is usually limited by weather and orchard conditions to a period of 1 to 2 weeks before budbreak. Moreover, the growth cycle of the fungus in that area is almost perfectly adapted to the use of an eradicant spray during that period. Sporodochia of *Sclerotinia laxa* apparently are formed in Door County only in the spring. In California and Oregon they may be formed soon after the fall rains begin, but this phenomenon has not been observed in Wisconsin, where sporodochial formation begins after the spring rains start (in 1942–1944 about 7 to 10 days before the buds began to swell) and continues for a month or more.

The advisability of using two special sprays, rather than one, for the control of *Sclerotinia laxa* is indicated by the data from the 1943 and 1944 experiments. The chief object of the dormant eradicant spray under Wisconsin conditions is the suppression of the fungal sporodochia, though it doubtless retains some protectant fungicidal value at blossom time. The bloom spray is chiefly protectant in nature, but it has the further purpose of lessening the danger of spray injury from the dormant application. The possibility of using the bloom spray alone has been considered. This practice has been followed for many years, often with good results, in Europe and western North America. Unfortunately, however, under Wisconsin conditions a single spray application during bloom does not insure good control of *S. laxa* in epidemic seasons.

Suggested measures for the control of *Sclerotinia laxa* on sour cherry under conditions such as we have encountered are: (a) Apply a dormant tree spray of copper-lime-monocalcium arsenite plus fish oil, 3–2–2 (1½ pints fish oil)–50, shortly before budbreak, and (b) apply a spray of Bordeaux mixture, 3–4–50, soon after the blossoms have started to open.

SUMMARY

Many isolates of *Sclerotinia laxa* from sour cherry in Door County, Wisconsin, were grown in culture and compared with isolates obtained from various hosts from the Pacific Coast States. General colony characters were lobing, zonation, and scant conidial production.

Conidia formed in most isolates on Emperor grapes within 3 days at 16° to 24° C., or on potato-dextrose agar within 2 months at 4° to 8° C. Conidia germinated readily at 16° to 32° C. Germination occurred at 4° and 34.5°, but not at 37° C. The minimum time for germination was 2 hours. When sufficient nutrients were present, the germ tubes were crooked and branched.

The optimum temperature for radial growth on potato-dextrose agar was about 28° C. Some growth occurred at 4° and 32°, but none at 37° C.

The relationship of temperature to infection and disease development on small, potted, sour cherry trees was studied under controlled conditions in the greenhouse. Infection and blighting of inoculated blossoms were most rapid at 24° C., but occurred at 5° and 30° C. Eleven degrees centigrade was more favorable for spur blight development than 16° C. or higher.

Most blossom infections originated either through the anthers or the stigmas, although occasionally other blossom parts, especially petals, were attacked. Filaments and sepals were infected only through wounds or after contact with rotted tissue.

Blossom time was established as the critical period for infection, though under experimental conditions unopened, white-tipped buds were frequently infected. Infection of green fruits and leaves sometimes occurred under especially favorable conditions in the greenhouse and in the orchard.

No important difference in susceptibility between Early Richmond and Montmorency sour cherries was apparent in the greenhouse, although spur blight had been observed to be much more severe on Early Richmond in the orchard.

Experimental sprays for the control of *Sclerotinia laxa* were applied over a period of 3 years, and excellent control was obtained from certain programs. In the epidemic season of 1943 the eradicant spray of copper-lime-monocalcium arsenite plus cold-pressed menhaden fish oil, 3–2–2 (1½ pints fish oil) –50, applied just before budbreak, gave satisfactory suppression of sporodochia and 97 per cent reduction in incidence of spur blight. Ninety-nine per cent reduction of spur blight was obtained when this eradicant spray was followed early in bloom by a protectant spray of

Bordeaux mixture, 3–4–50. The bloom spray alone did not affect the production of sporodochia, but it reduced the incidence of spur blight 82 per cent.

In Wisconsin thorough applications of a dormant and a bloom spray are suggested for effective control of *Sclerotinia laxa* on sour cherry.

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A STUDY OF THE SUGAR BEET SEEDLING DISEASE IN OHIO

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INTRODUCTION

The black root disease of sugar beet seedlings is one of the most wide-spread and economically significant diseases of plants. It occurs wherever beets are produced on a commercial scale. Coons and Stewart (6), who have investigated the majority of the beet-producing regions of the United States, report yearly, national losses due to this disease ranging from a trace to 25 per cent of the planted acres. Buchholtz (2) writes that the disease constitutes the chief limiting factor in obtaining maximum stands and yields. Campbell (3) in 1940 reported the disease present in all fields he had examined in Washington, infection ranging from a trace to 95 per cent of the stand. He considers this disease to be one of the most important factors in sugar beet production.

In Ohio, black root of sugar beet seedlings is the major factor in determining the number of acres planted and harvested. The losses in this State closely approximate those reported by Coons and Stewart for the total sugar beet area.

Although the average annual loss to the state and national economy is great, the actual importance of this disease to the industry is best appreciated when it is realized that individual fields may have a 100 per cent loss in seedling stand with a consequent depressing effect on future acres planted. Despite the high cash value of the beet crop, the farmer is reluctant to plant his fields to beets when faced with the possibility of a complete loss of the crop. Labor is still another problem. Until black root can be controlled, hand labor will be required for the blocking and thinning operations since the machines used for these operations depend upon an almost perfect seedling stand.

In any root rotting disease, four possible means of control are available. First, the agents responsible for the disease may be eliminated from the soil in the immediate vicinity of the plant by the use of seed-borne fungicides or the soil may be treated directly with fungicidal or fungistatic agents. Second, soil amendments may be utilized to change the physico-chemical nature of the soil, which may in turn affect the development of the disease. Third, varieties of the host plant may be developed which are resistant to infection. The fourth possibility, which may be related to the use of soil amendments, involves a change in the soil microflora with a resultant biologic antagonism between non-pathogenic soil organisms and those responsible for the disease.

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If any of these control measures is to be successfully developed it is first necessary to definitely establish the identity of the organisms causing the disease. Then investigations can be made of the physiology, morphology, and life cycle of the pathogen together with its relationship to the host plant and to other organisms of the soil.

Thus, this study was undertaken to determine the identity of the organisms responsible for the disease and the relative frequencies of their occurrence in diseased seedlings in Ohio.

A second objective of the work was to determine what effect, if any, applications of high phosphate content fertilizers had on the incidence of disease. Observations of commercial beet fields had indicated that in some instances the root rot disease was less severe in fields that had received high phosphate applications.

PERTINENT LITERATURE

The parasitic nature of this sugar beet seedling disease was first implied in 1890 when Hellriegel (15) found that by soaking the sugar beet seed ball in a one per cent solution of carbolic acid the amount of black root was significantly lessened. The knowledge regarding the organisms causing this disease was increased by Frank (12) and Kruger (17) who demonstrated that *Phoma betae* (Oud.) Fr. could cause a sugar beet seedling root rot. In 1901 Duggar and Stewart (8) isolated strains of *Rhizoctonia solani* Kühn from diseased sugar beet seedlings. In inoculation experiments with this organism the disease was reproduced in seedling beets. In 1906 Peters (33) described a seedling root rot caused by *Pythium debaryanum* Hesse and differentiated between the symptoms brought about by this organism and those due to infection by *Phoma betae*. Another of the agents of the disease was described in 1915 by Edson (9) when he isolated a previously undescribed fungus which he named *Rheosporangium aphanidermatus* (Edson) Fitz. and which was subsequently assigned to the genus *Pythium* and is now known as *Aphanomyces cochlioides* Drech.

The importance ascribed to these organisms as causal agents of the disease and to other fungi which have been considered responsible for black root is indicated by the following: Edgerton and Tims (10) have reported that *Rhizoctonia solani* infection caused losses up to 50 per cent of the crop and that *Phoma betae* was of minor importance in Louisiana. Campbell and others (4) isolated numerous fungi from beet seedlings in Washington but only *Phoma betae* isolates were pathogenic. Maxson (19) in isolations from 268 diseased seedlings found that *Fusarium* spp. constituted 53.36 per cent, *Phoma betae* 38.05 per cent, *Pythium* spp. 5.6 per cent, and *Rhizoctonia solani* 2.98 per cent of the isolations. In another series of observations he examined 970 diseased plants and found 70.93 per cent were infected with *Rhizoctonia solani*.

Reddy (21) has indicated that *Pythium debaryanum* was the principal cause of the disease while *Phoma betae* was of little importance. Buchholtz

(2) has reported that *Pythium debaryanum* caused at least 95 per cent of sugar beet seedling root rot damage. Coons, Kotila, and Bockstahler (5) have regarded *Aphanomyces* spp., common in all agricultural soils, as important causal agents of the disease. Mélhus et al (20) found *Aphanomyces cochlioides* in only three of the fields examined by them. Hodges (16) isolated two species of *Fusarium* which he reported causing 50 to 75 per cent of all sugar beet seedling infections. From Europe, Esmarch (11) has reported *Phoma betae* as the primary cause of the disease with *Pythium debaryanum* ranking second and *Aphanomyces levis* DeBary third, the ratio of their occurrence being roughly 7–4–2. He also reported *Alternaria tenuis* Nees., and *Macrosporium cladosporioides* Desm. causing a seedling root rot. Greis (13, 14) has written that *Alternaria tenuis* spreads through the seed and attacks it saprophytically, later developing as a facultative parasite on the seedling. Marchal (18) has reported that the disease, in Belgium, is caused by *Bacillus mycoides* as well as by *Phoma betae, Pythium debaryanum,* and *Rhizoctonia solani.*

These various and, in some instances, contradictory reports concerning the fungi responsible for the disease and the relative importance of each of them as causative agents seemed to need clarification before an adequate control program could be developed for Ohio.

SYMPTOMS OF THE DISEASE

The black root disease of sugar beet seedlings has been divided into three distinct types. First, a preemergent type which, as the name implies, results in the death of the plants immediately after seed germination and prior to the emergence of the seedling from the soil. Second, a damping-off form of the disease which is apparent from the time of emergence of the seedling to the time when the first true leaves develop. In this type of the disease, the plants are attacked when the tissues are still rather tender and succulent. Infection of the beet seedling at this stage results in the complete wilting and immediate death of the plant. In many instances the disease develops so rapidly that the plant collapses at the base of the hypocotyl and falls over before the cotyledons wilt. A water-soaked, brown, discolored area extends up and down the hypocotyl or the upper portion of the young taproot from the point of entry of the pathogenic organism. In later stages this discoloration extends well up into the petioles of the cotyledons. The collapse of the hypocotyl of the seedling is followed within a very short time by the desiccation of the seedling. The hypocotyl of the seedling shrinks to an inconspicuous, dark, slender thread. The rapidity with which these events occur may be an explanation for the failure on the part of commercial growers in particular to recognize the large toll taken by this disease.

The third type of the disease is by far the most conspicuous of the three. The affected seedlings are not immediately killed and usually continue to live for several days after the initial symptoms appear. Fre-

quently such diseased plants recover and persist through the growing season. In these cases the tap root is replaced by numerous lateral roots. This lowers the quantity as well as the quality of the crop, because, although these lateral roots become fleshy, their total weight rarely equals that of a single normal beet root. The beets with numerous fleshy lateral roots have a lower sugar content and higher impurity content than do the larger single roots. The relatively high impurity content makes refining more difficult and the low sugar content naturally decreases the amount of recoverable sugar per ton of beets. This type of black root appears after the beets have developed beyond the early seedling stage. The beets that are infected after the first pair of true leaves are well developed frequently do not die. By this time secondary tissues have developed and prevent the complete destruction of the seedling. The hypocotyl or developing crown of the beet is composed essentially of intricately anastomosing vascular bundles containing lignified cells and is nearly free of parenchymatous cells (1). The name black root is particularly applicable to this type of the disease because of the general blackening of the hypocotyl which begins slightly below the ground and extends up into the cotyledons. The leaves of a plant so affected may develop an abnormal downward curvature but remain green and turgid.

METHODS AND MATERIALS

A majority of the diseased seedlings from which isolations were made were obtained from commercial fields although some were made from sugar beet seedlings growing in the greenhouse. Greenhouse seedlings were used for isolation work before field-planted beets were available and at various times during the summer when soil was removed from fields in which root rot was severe. The soil used in these studies was obtained from the beet-producing areas of the State.

The commercial fields from which specimens were collected were planted at irregular intervals from May 25 to July 1. Collections were made at weekly intervals beginning when only a few seedlings had emerged from the soil and continuing until the plants were well beyond the seedling stage.

The study of any plant disease is facilitated if the pathogens responsible for the disease can be isolated from the affected plant tissue by direct plating on a solid medium. For this to be successful it is either necessary to sterilize the surface of the diseased tissue without causing the death of any organisms within the tissue or to use a selective medium that will prevent or retard the growth of contaminating organisms. The environmental association of sugar beet roots with the fungi and bacteria of the soil make this goal difficult to attain. Many types and combinations of sterilizing agents were tested at varying concentrations and used in conjunction with various media. After numerous trials it was found that the sterilizing agents either killed all organisms present or failed to kill the nonpathogenic or saprophytic organisms associated with the beet root surfaces.

An additional difficulty was recognized when it became apparent that several organisms were involved, differing widely in characteristics and in susceptibility to sterilizing agents. Attempts to find a differential medium were equally unsatisfactory.

In order to obtain the cultures for this study a method described by Drechsler (7) was modified and used, with few exceptions, throughout the period during which diseased beets were collected.

Depending on the size of the seedling, the seedling without the leaves, the roots alone, or the hypocotyl, was washed thoroughly in tap water. The part used was then rinsed twice in sterile water and placed in approximately 20 cc. sterile tap water contained in a cotton-plugged tube. These were incubated for 24 to 48 hr. at room temperature then examined for the presence of mycelial growth surrounding the section of diseased seedling. All those on which such growth was not present were discarded. The sections surrounded by mycelial growth were removed from the tubes and blotted between folded pieces of sterile paper toweling. This removed the excess water and with it a large percentage of the bacterial contaminants. After blotting, the beet fragments were placed on corn meal agar medium in Petri dishes. After another incubation period of 24 to 48 hr. a section of agar was cut from the periphery of the developing colony. When this was transferred to potato-dextrose agar slants pure cultures would usually result.

Despite the superiority of this method of obtaining pure cultures it was considered possible that the actual relative numerical frequencies of the occurrence of the pathogens was not being shown by the isolation technique used. For this reason the following procedure was devised.

Collections of seedlings were made in the field by taking up the plants with the surrounding soil. A trowel was used to make a circular cut 6 to 8 inches into the soil at a distance of 2 to 3 inches from the beet. The plants were then lifted, an effort being made to keep the roots as nearly intact as possible. The plants and the adhering soil were wrapped in wax paper and taken to the laboratory where they were placed in water and the soil carefully removed from the roots. By doing this, comparatively few of the small lateral rootlets were broken. After the roots had been well cleaned they were floated in water in one half of a Petri dish and examined with the aid of a dissecting microscope. When lateral roots with some discoloration were observed they were removed in lengths of 3 to 5 cm. and placed in sterile tap water for additional washing. These fragments of roots were washed thoroughly in three changes of sterile distilled water and placed in a drop of sterile distilled water in the center of a vaseline-ringed cover slip. The cover slip was then inverted over a Van Tiegham cell mounted on a slide or over a deep, hollow ground slide. The completed preparations were placed in a large moist chamber and incubated at room temperature for 24 hr.

The growth characteristics of the organisms associated with the sugar beet seedling diseases in Ohio, as indicated from isolations thus made, were sufficiently distinctive to permit ready identification when the hanging drop preparations were examined microscopically.

In all pathogenicity tests sterile Brookston clay soil was mixed with cultures growing on the following medium: equal parts, by weight, of corn meal and sand were mixed, and the amount of the mixture that could be contained in a 100-cc. beaker was placed in a 200- or 300-cc. flask. One hundred ml. of water were then added to each flask. The flasks were plugged with cotton and autoclaved at 15 lb. pressure for two hours on two consecutive days.

Transfers were made from actively growing cultures of the fungi, on potato-dextrose agar slants, to the corn meal and sand medium. The mouths of the flasks were then covered with waxed paper to lessen the drying out of the medium and the cultures were incubated for 10 to 14 days. Following incubation the mycelium of the fungi had usually thoroughly permeated the medium. The entire mycelial culture was then removed from the flask and mixed into the soil at the rate of one such culture to two 6-inch pots of soil. After the soil was mixed with the culture it was well watered and left undisturbed for three days, after which time it was seeded with sheared seed of the type used in commercial plantings in Ohio.

The soil temperature studies were made in Wisconsin type temperature tanks.

RESULTS

Using the method of isolation previously described 689 cultures were obtained. The species distribution of the isolations is shown in table 1.

Because of the large number of isolates it was considered advisable to test pathogenicity of representative samples from each of the groups rather than test all of the isolates. In the preliminary test, any organism causing a reduction in the number of seedlings to emerge from the soil or any definitely diseased seedlings was selected for a second and more definitive test. In the preliminary test no attempt was made to reisolate the causative fungi from the diseased seedlings. Such reisolations were made from beets becoming diseased in the second test.

TABLE 1.—*Distribution of 689 isolations among species*

Organism	Times isolated	Percentage of total
1. *Aphanomyces cochlioides*	111	15.9
2. *Pythium debaryanum*	87	12.6
3. *Rhizoctonia solani*	52	7.6
4. *Alternaria tenuis*	22	3.2
5. *Fusarium* spp.	280	40.6
6. *Papulospora* sp.	30	4.3
7. Undetermined Phycomycetes	27	3.9
8. Unidentified	80	11.7

In all of the pathogenicity tests the soil was mixed with cultures of the fungi as previously described. The seed, sheared and untreated, was planted at the rate of 25 seed pieces to each 6-inch pot of soil.

After the seedlings had emerged from the soil they were examined fre-

TABLE 2.—*The pathogenicity of fungus isolates to sugar beet seedlings*[a]

Organism and isolate number	Plants emerging	Reduction in emergence based on mean of controls	Post emergence deaths	Plants alive at end of test	
Aphanomyces cochlioides	Number	Number	Number	Number	Per cent
1	12.0	14.6	12.0	00.0	00.0
2	13.0	13.6	13.0	00.0	00.0
3	15.5	11.1	15.5	00.0	00.0
4	12.5	14.1	1.0	11.5	43.2
5	17.5	9.1	6.0	11.5	43.2
6	18.0	8.6	3.5	14.5	54.5
7	18.0	8.6	3.0	15.0	56.3
8	19.5	7.1	3.0	16.5	62.0
9	19.0	7.6	2.0	17.0	63.9
10	23.5	3.1	1.0	22.5	84.5
Average	16.8	9.7	6.0	10.8	40.8
Pythium debaryanum					
1	4.5	22.1	1.5	3.0	11.2
2	4.5	22.1	0.0	4.5	16.9
3	9.5	17.1	3.5	6.0	22.5
4	10.5	16.1	1.5	9.0	33.8
5	16.5	10.1	2.0	14.5	54.5
6	16.0	10.6	4.5	11.5	43.2
7	19.0	7.6	1.5	17.5	65.7
8	24.5	2.1	6.5	18.0	67.6
Average	13.1	13.5	2.6	10.5	39.4
Rhizoctonia solani					
1	00.0	26.6	00.0	00.0	00.0
2	6.5	20.1	0.0	6.5	24.4
3	9.5	17.1	0.0	9.5	35.7
4	19.0	7.6	10.5	8.5	44.7
5	21.5	5.1	2.0	19.5	73.3
6	21.5	5.1	0.0	21.5	80.0
Average	13.0	13.6	2.0	10.9	43.0
Unidentified Phycomycetes					
1	5.0	21.6	2.5	2.5	9.3
2	15.0	11.6	5.0	10.0	37.5
3	22.5	4.1	5.0	17.5	65.7
4	27.5	− 1.0	1.0	26.5	103.0
Average	17.5	9.6	3.3	14.1	53.9
Control	26.6	0.0	0.0	26.6	100.0

[a] Figures based on the average of two pots per isolate except for the control which is an average of eight pots.

quently for indications of disease. The tests were culminated three weeks after seedling emergence. At that time counts were made of the apparently healthy plants and of the seedlings which had been removed because of disease. By totaling these two figures and comparing them with the number

of seedlings in the check pots the amount of preemergent disease could be determined.

Table 2 is a list of all isolates tested and their degrees of virulence. The results given are the averages from two inoculated pots for each isolate.

As would be expected, from the results of previous investigations, the various isolates of *Rhizoctonia solani* show varying degrees of virulence. This variation is undoubtedly related to the differing physiologic strains within the species. It is also illustrated by the results of this test that there are wide variations in virulence among the ten isolates of *Aphanomyces cochlioides* tested. A similar result is indicated for *Pythium debaryanum*, as well, but here the range or spread of pathogenicity is not so great as with *A. cochlioides*. In both instances it would seem that the differences between the least virulent and the most virulent are sufficiently great that

TABLE 3.—*Fungi identified from hanging drop cultures of sugar beet seedling tissues*

Date[a]	*Aphano-myces cochlioides*	*Pythium*	*Rhizoc-tonia*	*Fusarium*	Unidenti-fied	Total
May 30	15	4	4	2	0	25
June 7	28	4	0	0	0	32
June 12	14	6	2	3	2	27
June 18	10	5	1	0	2	18
June 22	17	6	0	1	0	24
July 1	5	5	3	3	4	20
July 10	24	8	0	2	3	37
July 24	21	5	7	0	1	24
July 30	12	2	5	1	0	20
Total	146	45	22	12	12	210
Percentage of total	69	21	10	5	5	

[a] Approximate.

they can not be explained by the extremely slight environmental differences among the various units of this test.

Table 3 is a summation of the identifications made directly from the hanging drop preparations. No attempts were made to isolate pure cultures from these preparations.

Observations of field planted beets having indicated that high phosphate applications seemed to result in the reduction of the amount of black root in some plantings, one of the more virulent isolates of the *Aphanomyces cochlioides* was selected to study the effects of varying temperatures and phosphate applications on the development of the disease.

Sterilized Brookston clay soil contained in Wisconsin type temperature tanks was mixed with 10-day-old cultures of *Aphanomyces cochlioides* growing on potato-dextrose agar plates. The soil was well watered and left undisturbed for three days. It was then planted with seed of the type used in the pathogenicity tests and phosphate was added at the rates of 400 and 500

lb. super phosphate per acre. There were two containers of each treatment at each temperature and two check containers (no fertilizer) at each of the five temperature levels used in the test. The averaged results are in table 4.

TABLE 4.—*Effect of temperature and phosphate applications on the development of black root of sugar beet seedlings*

Temperature in degrees C.	Percentage of beets dead in relation to the total number emerged in checks		
	No fertilizer	(Super-phos. 400 lbs.)	(Super-phos. 500 lbs.)
32	89.5	44.9	86.4
28	58.5	86.1	74.4
24	97.0	78.4	64.0
20	66.6	12.0	30.9
18	50.0	8.1	11.3

SUMMARY AND CONCLUSIONS

Based on the number of times isolated, the fungus primarily responsible for black root of sugar beets, in Ohio, is *Aphanomyces cochlioides* Drech., the isolates of this fungus constituting 15.9 per cent of a total 689 isolations. This fact was amply substantiated by observations made by identifying the organism in hanging drop preparations. Of 210 identifications made in this way 69 per cent were *Aphanomyces cochlioides*. *Pythium debaryanum* Hesse appeared in culture with a frequency almost equal to that of *Aphanomyces cochlioides* but in the hanging drop preparations the latter was identified 146 times to 45 times for *Pythium debaryanum*. Third in frequency of isolation and identification as a pathogen was *Rhizoctonia solani* Kühn: 7.6 per cent of the isolations and 10 per cent of the hanging drop cultures were identified as this species. Some of the isolates of *Rhizoctonia solani* were among the most virulent pathogens.

Seasonal variation in the organisms isolated was negligible. During the season the ratio of one fungus isolated to another was remarkably constant.

From the temperature tank phosphate experiment it can be concluded that the amount of black root due to *Aphanomyces cochlioides* tends to increase when soil temperatures rise from 18° to 24° C. and are higher despite phosphate applications at temperatures of 24°, 28°, and 32° than at 20° and 18° C.

At 28° and 32° C., phosphate applications have no effect in reducing the amount of black root. However, at soil temperatures of 18°, 20°, and 24°, both 400- and 500-lb. applications caused a marked reduction in the number of seedlings becoming diseased.

This fertilizer effect at low temperatures is highly significant from a practical standpoint because these are the temperatures that prevail during the early spring when most of the beet acreage is being planted.

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A DISEASE OF CABBAGE AND OTHER CRUCIFERS DUE TO CERCOSPORELLA BRASSICAE[1]

P. W. MILLER[2] AND F. P. McWHORTER[3]

During the late winter and spring of 1943–1944 the foliage of cabbage being grown for seed in Coos and Curry Counties, Oregon, was infected with a *Cercosporella* sp. hitherto unreported on this host in Oregon. Bird rape (*Brassica campestris* L.), known locally as wild mustard, and turnip (*Brassica rapa* L.) growing close by were found abundantly infected with the same or a closely related organism.

A brief description of this disease on cabbage in Oregon has already been published in a preliminary report (15).

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Leaf spots of *Brassica* spp. due to Cercospora-like fungi are widely distributed. They have been reported from the following countries: Brazil (22), England (5, 7, 20, 22), France (22), Guam (22), Puerto Rico (25, 26), China (13), United States (1, 25), and Yugoslavia (22). In the United States, leaf spots of *Brassica* spp. due to Cercospora-like fungi have been reported from the following states: Alabama (25), California (11, 21, 25), Connecticut (1, 25), Delaware (25), Florida (1, 24, 25), Georgia (25), Illinois (1, 25), Indiana (3, 25), Louisiana (2, 25), Massachusetts (8, 25), Maryland (25), Minnesota (25), Mississippi (17, 25), Missouri (14, 25), New Hampshire (25), New Jersey (25), New York (25), Oklahoma (25), Oregon (6, 15, 25), Pennsylvania (16, 25), South Carolina (25), Texas (1, 12, 25), and Virginia (25).

The disease on cabbage has so far been reported only from the following states: Alabama (25), California (21), Mississippi (17), and Oregon (15). It also has been reported from Puerto Rico (25, 26).

ECONOMIC IMPORTANCE OF THE DISEASE

The disease on cabbage has not been of major importance in Oregon. Infected plants have not been damaged severely, as the disease was confined to the lower leaves. It did not attack the inflorescences. However, the causal fungus has caused economic damage to mustard and turnip being

FIG. 1.   Young lesions on a cabbage leaf caused by *Cercosporella brassicae*.   Note dendritic type of lesion.

grown for greens in other regions (2, 24).   Moreover, damage to culti-vated turnip and mustard plants being grown for seed production has re-sulted from a reduction in the functional leaf area.

SYMPTOMS

Cabbage.   Only the leaves of the cabbage plant have been attacked by this fungus.   In its initial stages, the disease superficially resembles downy mildew of cabbage due to *Peronospora parasitica*, the young lesions being small, averaging approximately 2 mm. in diameter, dark gray or black, and dendritic in form (Fig. 1).   The lesions later lose their dendritic con-figuration and become more or less rectangular or rounded with well-de-fined margins.   The mature lesions measure from 10 to 22 mm. in diameter and are ashy black.

FIG. 2.   Lesions on turnip leaf caused by *Cercosporella brassicae.*

Turnip and wild mustard. The lesions on turnip and wild mustard leaves are conspicuous, typically round, semi-transparent, with brownish-gray centers and well-defined brown or tan margins (Fig. 2) ; they are larger than those on cabbage, averaging about 30 mm. in diameter when fully developed. An extreme necrosis of the invaded tissues occurs. The lesions do not have the dendritic form so characteristic of the disease in its initial stages on cabbage.

PATHOLOGICAL HISTOLOGY

The fungus mycelium in both cabbage and turnip leaves is predominately intercellular.

In turnip, the protoplasts and cell walls in the invaded areas are destroyed which results in a necrotic type of lesion. In cabbage, however, killing of the cells is more restricted. The paths of the lateral hyphae are demarked by black, necrotic masses formed from the middle lamella and abutting cell wall (Fig. 3, C). The adjacent cells are partly emptied, but the protoplasts of those farther away from the advancing hyphae are only yellowed with masses of lipoid elements present as free oils or held in large vacuolate elaioplasts. The localization of killed areas accounts for the dendritic pattern of the young lesions.

THE CAUSAL ORGANISM

Morphology

The conidia of the causal fungus from lesions on cabbage vary at maturity from 70 to 100 $\mu \times 2$–3 μ, the average dimensions being 80 $\mu \times 2.5 \mu$. They are hyaline, elongate-cylindrical, mostly 3- to 4-celled, with indistinct cross walls. The young conidia are more or less straight but mature conidia are typically curved at both ends (Fig. 3, A). The conidia are not borne on a stroma but originate from hyphal strands loosely aggregated beneath the stomata. They are commonly borne uniformly over the surface of the lesion.

Conidia from lesions on wild mustard and turnip are similar to those of the fungus on cabbage except that they are irregularly partitioned, thus having one cell of smaller dimensions than the others. On freshly collected material, the conidia contain from 3 to 5 cells and average 65 $\mu \times 2.5 \mu$ (Fig. 3, B). As in the lesions on cabbage, the conidia are not borne from a stroma but from hyphal strands which generally are massed within the stomatal openings.

Taxonomy

From its conidial characteristics the causal fungus is evidently *Cercosporella brassicae* (Fautr. et Roum). Hoehnel (18, 23), a synonym for which is *Cercospora (Cercosporella) albo-maculans* Ell. et Ev. (9, 19).

Host Range and Pathological Significance

Cercospora-like fungi have been reported on the following Crucifers: *Brassica oleraceae* var. *capitata* L. (1, 13, 21, 25), *B. oleraceae* var. *italica*

Plenck. (3, 14, 25), *B. oleraceae* var. *viridis* L. (6, 25), *B. chinensis* L. (11, 22), *B. campestris* L. (8, 13, 25), *B. juncae* Coss. (25), *B. nigra* L. Kock (25), *B. napus* L. (25, 26), *B. kaber* (D. C.) L. C. Wheeler (25), *B. oleracea* var. *gemifera* D. C. (11), *B. campestris* var. *napobrassica* (L.) D. C. (18,

FIG. 3.   A and B, Conidia of *Cercosporella brassicae:* A, from lesions on cabbage leaf, × 200; B, from lesions on turnip leaf, × 200; C, Photomicrograph of young lesion on cabbage leaf showing the effect of the destruction of cell walls, × 75.

25), *B. pekinensis* (Lour.) Rupr. (1, 25), *B. rapa* L. (1, 24, 25, 26), and *Raphanus sativus* L. (4, 10, 14, 25).

Despite the widespread occurrence of Cercospora-like fungi on many Crucifers, there are relatively few reports of these fungi causing economic

damage. In 1927, Davis (8) tried to infect beets, celery, parsnips, carrots, and cabbage by inoculation with conidia of a Cercospora-like fungus from lesions on Chinese cabbage but without success. Weber (24) reported that in Florida *Cercosporella albo-maculans* was widespread and common on turnip, but not severe enough to cause any damage except in certain instances when it prevented turnip greens from being marketed. Chupp, in a personal communication, stated that the fungus was rather prevalent and sometimes destructive on turnip in New York. In 1943, Snyder and Baker (21) reported a leaf spot on cabbage in California due to *Cercospora* sp. but the disease was of little economic significance, being confined to the lowest leaves on the plant. In 1944, Baines (3) reported leaves of broccoli (*Brassica oleracea* var. *italica*) were severely infected with *Cercosporella albo-maculans* (E. & E.) Sacc.

Cross Infection Studies

The occurrence of this disease on cabbage, wild mustard, and turnip growing in close proximity suggested that wild mustard or turnip was the likely source of the disease on cabbage. To determine this, cross inoculations under controlled greenhouse conditions were performed using conidial suspensions from sporulating lesions on cabbage and turnip, respectively. This procedure was necessitated by the failure of the causal organism to fruit in culture. Numerous lesions indistinguishable from those on turnip in nature were produced on leaves of young turnip plants by atomizing them with suspensions of conidia from sporulating lesions on cabbage. Conversely, typical lesions were produced on leaves of young cabbage by atomizing them with suspensions of conidia from sporulating lesions on turnip. These results indicate that the disease on these two hosts has a common cause.

SUMMARY

A disease of cabbage and other Crucifers caused by the fungus *Cercosporella brassicae* (Fautr. et Roum.) Hoehnel is described and discussed.

The geographical distribution, economic importance, and host range of the disease are discussed.

The causal fungus and its effects on host cells are described.

Cross-inoculation studies indicate that the disease on cabbage, wild mustard, and turnip has a common cause.

U. S. DEPARTMENT OF AGRICULTURE
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AN ANTIBIOTIC SUBSTANCE ACTIVE AGAINST CERTAIN PHYTOPATHOGENS [1]

CURT LEBEN AND G. W. KEITT [2]

During the past few years the marked advances in the use of antibiotic substances in the field of animal disease control have stimulated research on the use of these agents in the control of plant diseases incited by bacteria or fungi (e.g., 1, 2, 4, 6, 7, 8, 9, 10, 13, 15, 16). In this connection, this laboratory has published notes on an unidentified antibiotic from an actinomycete, which was effective as a protectant spray against apple scab and tomato early blight in greenhouse tests (11, 12). The purpose of the present paper is to report more fully studies on the production, purification, properties, and *in vitro* biological activity of this material. Investigations on its possible use in the control of plant diseases are being extended and will be reported later.

The antibiotic has tentatively been given a name, antimycin, since on the basis of properties and biological effects described in the present paper it appears to be distinct from all others that have come to our attention.

THE ANTIBIOTIC ORGANISM

In 1945, a plate culture of *Venturia inaequalis* (Cke.) Wint. was markedly inhibited by a white, slow-growing actinomycete contaminant. This organism produced the musty odor typical of actinomycetes, and in old potato agar[3] cultures a small amount of a green-black pigment was noted. According to the classification of Waksman and Henrici (17) the organism has been placed as an undetermined species in the genus *Streptomyces* (See figure 1,-A).

Several modes of culture have been used to grow the antibiotic organism, and in each antimycin was produced. Temperatures employed in the various experiments ranged from 24° to 28° C. On nutrient agar or potato agar, growth was satisfactory and the active substance readily diffused through the medium. In continuously shaken liquid substrates such as a corn steep liquor medium[4] the organism grew vigorously as a heavy suspension of spores and hyphae. The maximum antimycin concentration in the culture filtrate for this method of growth was reached in three or four days. During this time there was a reaction change from pH 7.0 to pH 8.0–8.5.

[3] Per liter: extract from 40 gm. of sliced, peeled potatoes steamed one half hour in 500 ml. of water; glucose, 5 gm.; agar, 17 gm.

[4] Per liter: corn steep liquor (50 per cent solids), 50 gm.; glucose, 10 gm.; and CaCO₃, 1.5 gm. The medium was adjusted to pH 7.0 before the CaCO₃ was added.

The organism also has been grown in 14-liter lots of corn steep liquor meddium in stationary tanks provided with stirrers and means for continously passing sterile air through the medium.[5] Shake flask fermentations have given yields of 1–3 units (a unit is defined later in this paper) per ml. of the culture filtrate; in tank fermentations yields of 3–5 units per ml. have been obtained.

The organism has been kept for over two years in soil or under oil (14) with apparent stability.

ASSAY, PARTIAL PURIFICATION, AND SOME PROPERTIES OF THE ACTIVE MATERIAL

A blotting paper disc assay using the fungus *Glomerella cingulata* (Stoneman) Spauld, and v. Schrenk., our isolate number 22, was employed to determine the potency of antimycin in various preparations. In preliminary tests this isolate gave the most satisfactory type of inhibition rings in trials using various temperatures, media, and a number of fungi that were inhibited by the culture filtrate. For the assay, 20-ml. amounts of nutrient agar were allowed to harden in Petri plates. These were then layered with five ml. of a spore suspension of the assay organism in warm (40°–45° C.) nutrient agar. Blotting paper discs 6 mm. in diameter were dipped in the preparation to be assayed, drained in a uniform manner, placed on the surface of each of three seeded plates, and the plates incubated 24 to 36 hours at 28° C. On each plate usually five different test preparations and a standard solution of one unit per ml.[6] were tested at one time. The diameters of the resulting zones of inhibition were measured and the potency of a given preparation determined with the aid of a dilution curve of the standard. The diameter of the zone of inhibition for a given preparation is a straight line function of the log of the concentration. The assay has not been evaluated statistically; however, we have found it useful for the type of studies that will be described.

The antibiotic in the culture filtrate was partly purified by means of the method indicated in table 1. Cultures were prepared by heavily seeding 300-ml. amounts of the corn steep liquor medium in liter Erlenmeyer flasks, which were shaken in a reciprocating shaker for four days at 26° C. Antimycin has also been extracted from the culture filtrate with butanol, although in our hands this method was not so efficient as that given in table 1.

Ethanol solutions prepared as indicated in table 1 were deep red and had the earthy odor typical of actinomycetes. On the addition of four to nine volumes of water, an amorphous precipitate was formed that contained all, or nearly all, of the activity. From this precipitate antimycin was extracted with water at pH 9.3 and partly extracted with water at

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[5] For these tests the tank fermentation equipment of the Department of Biochemistry, University of Wisconsin, was used. Grateful acknowledgment is made to M. J. Johnson and W. E. Brown for aid and suggestions made in connection with this phase of the work.

[6] An ethanol solution of the active material, giving zones of inhibition from 16 to 18 mm., was arbitrarily assigned a potency of one unit per ml. and used as a standard. It has been kept 14 months at 8° C. with no apparent loss in activity.

pH 6.0; it was reprecipitated in an amorphous form by adjusting the pH to 2.5. At pH 9.3 no activity remained after two days at room temperature;
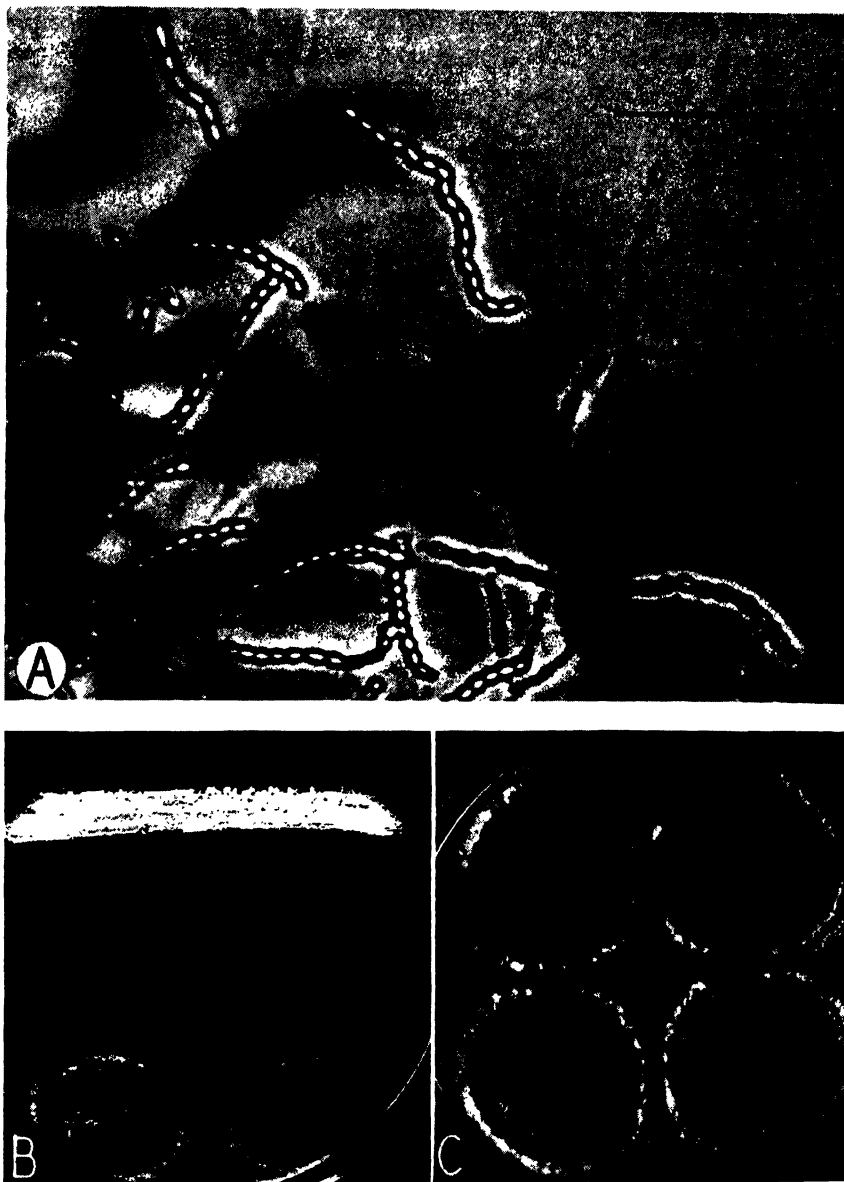
FIG. 1. A. Photomicrograph of aerial conidial chains of the antibiotic organi.m. B. *Sclerotinia fructicola* (Wint.) Rehm seeded 5, 20, and 35 mm. from a streak of the antibiotic organism. Note complete inhibition of *S. fructicola* at 5 mm. and reta led growth at 20 and 35 mm. C. Control plate containing *S. fructicola*.

at pH 11.0 no activity remained at the end of an hour. Antimycin in the culture filtrate (pH 8.3) was inactivated in eight minutes at 100° C. Water

suspensions at pH 2.5 and ethanol solutions appeared to be stable at 8°C. About one half of the activity in ethanol solutions was lost on refluxing 1.5 hours.

Antimycin was adsorbed by Norite from ethanol and from the culture filtrate; elutions with a number of reagents were not successful. Dialyzability was tested by sealing 25 ml. of the culture filtrate in a Visking cellophane bag, which was suspended overnight in a covered beaker containing 500 ml. of distilled water and a few drops of chloroform to act as a preservative. An assay indicated no change in potency of the residue in the bag; the dialysate, when concentrated to a small volume under reduced pressure, showed no activity. Aqueous suspensions at pH 6.1 (15 units per ml.) gave the following negative tests: Molisch, biuret, Hopkins-Cole, xanthoproteic, Millon, FeCl$_3$, and ninhydrin.

TABLE 1.—*Fractionation procedure*

Step	Treatment	Total volume	Total units	Units per mg. dry weight
		ml.		
I.	Culture centrifuged. Sediment discarded.	800	a	0.16
II.	Supernatant acidified to pH 2.5 (HCl). Suspension centrifuged. Inactive supernatant discarded. Precipitate extracted four times with 40-ml. amounts of absolute ethanol.	160	2,400	7.57

a Not assayed directly. Assumed to be 2,400 units since discarded supernatant in step II. was inactive.

When ethanol solutions prepared as indicated in table 1 were dried *in vacuo* and from one to four times the original volume of various solvents added and allowed to stand four to 12 hours, the maximum activity that could be accounted for in the various solvents was from 30 to 50 per cent of the original. Under these conditions reagents that dissolved the maximum amount of antimycin were acetone, butanol, chloroform, ethanol, ether, ethylene glycol, methanol, methyl acetate, propylene glycol, and pyridine. Antimycin was less soluble in benzene, carbon tetrachloride, petroleum ether, and toluene, and insoluble in water.

Fractionations have been carried further than indicated in table 1. These procedures, at present not fully evaluated, are being studied further and will be reported later.

THE ACTIVITY OF ANTIMYCIN IN VITRO

Screening Tests

In screening tests, the antibiotic organism was streaked near the side of plates containing nutrient agar (for bacteria) or potato agar (for fungi), and the plates incubated four days at 24° C. to permit growth of the organism and the diffusion of antimycin. Plates then were seeded with test organisms. Bacteria were streaked perpendicularly from the *Streptomyces*

culture, and fungi were seeded with small pieces of the mycelium placed at varying distances from the *Streptomyces* streak[7]. Inhibition was noted in two or more trials by the absence or reduction of growth adjacent to the antibiotic organism after an incubation period at a temperature suitable for the growth of the test organism (Fig. 1; B, C). All of the fungi except *Venturia inaequalis* were incubated four days at 24° C.; *V. inaequalis* was incubated 2 weeks at 16° C. The phytopathogenic bacteria were incubated two days at 24° C.; the remaining bacteria were incubated 24 hours at 37° C.

All the fungi tried in the screening tests were inhibited in varying degrees. The species tested were: *Alternaria solani* (E. and M.) Jones and Grout, *Ascochyta* sp. (from pea)[8], *Chalara quercina* Henry, *Cochliobolus* (*Ophiobolus*) *miyabeanus* Ito and Kuribay,*Colletotrichum circinans* (Berk.) Vogl., *C. graminicolum* (Ces.) G. W. Wils.[8], *C. lagenarium* (Pass.) E. and H., *C. lindemuthianum* (S. and M.) Bri. and Cav.[8], *C. pisi* Pat., *C. phomoides* (Sacc.) Chester, *Fusarium oxysporum* f. *conglutinans* (Wr.) S. and H., *F. oxysporum* f. *lycopersici* (Sacc.) S. and H., *F. oxysporum* f. *nicotianae* (Johns.) S. and H., *F. oxysporum* f. *pisi* (Sny.) S. and H., *Gibberella zeae* (Schw.) Petch, *Glomerella cingulata*[8], *Helminthosporium sativum* Pam., King, and Bakke, *Macrosporium* sp. (from muskmelon), *M.* sp. (from pea), *Mycosphaerella citrullina* (Sm.) Gross., *Neurospora crassa* Shear and Dodge, *Nigrospora sphaerica* (Sacc.) Mason, *Phoma lingam* (Tode) Desm., *P. terrestris* Hansen, *Phycomyces blakesleeanus* Burgeff, *Pythium graminicolum* Subr., *P.* sp., *Sclerotinia fructicola*, *S. laxa* Ader. and Ruhl., *S. minor* Jagger, *S. sclerotiorum* (Lib.) De Bary, *Stemphylium sarcinaeforme* (Cav.) Wilts., and *Venturia inaequalis*[8].

The bacteria used in screening tests were the following: *Agrobacterium tumefaciens* (Smith and Townsend) Conn, *Bacillus cereus* var. *mycoides* (Flügge) Bergey et al., *B. subtilis* Cohn *emend* Prazmowski, *Corynebacterium fascians* (Tilford) Dowson, *Erwinia amylovora* (Burrill) Winslow et al., *Escherichia coli* H 52 (Migula) Castellani and Chalmers, *Micrococcus pyogenes* var. *aureus* 209 P. (Rosenbach) Zopf, *Proteus vulgaris* Hauser. *Pseudomonas aeruginosa* (Schroeter) Migula, *P. savastanoi* (Erw. Smith) Stevens, *P. solanacearum* Erw. Smith, *Serratia marcescens* Bizio, *Xanthomonas beticola* (Smith, Brown, and Townsend) Bergey et al., and *X. campestris* (Pammel) Dowson. The only species that were affected were *B. cereus* var. *mycoides* and *E. amylovora*, which were completely inhibited 12 and 17 mm., respectively, from the *Streptomyces* culture.

Agar-streak Tests

With some of the organisms found to be inhibited in the screening tests, agar-streak tests (18) were made to determine if the material partly puri-

[7] For screening tests and the agar-streak tests, which are described later, inocula of all fungi except *Venturia inaequalis* were prepared from potato agar cultures that had grown four to seven days at 24° C. That for *V. inaequalis* was made from three- to five-week cultures kept at 16° C. Suspensions of the phytopathogenic bacteria were made from nutrient agar cultures that had grown 48 hours at 24° C.; the suspensions for the remaining bacteria were prepared from 24-hour cultures held at 37° C.

[8] More than one isolate tested.

fied on the basis of results with one assay organism was effective against other organisms, and, if so, to note the amount of the antibiotic substance necessary to inhibit the various organisms.

From a concentrate prepared as indicated in table 1, dilutions were made in sterile water. The resulting suspensions were mixed well in warm (40°–45° C.) potato agar, plates were poured, and the medium was allowed to solidify. Media containing the same amounts of ethanol as in dilutions

TABLE 2.—*The concentration of an antimycin preparation that prevented growth of various organisms in agar-streak tests*

Organism[a]	Isolate number	Inhibition point[b]
		µg. per ml.
Ascochyta sp. (pea)		25.0
Bacillus cereus var. *mycoides*	157	125.0
Do	158	125.0
Chelara quercina		0.8
Colletotrichum circinans		1.6
Do *lindemuthianum*		12.5
Do *phomoides*		0.8
Do *pisi*		1.6
Fusarium oxysporum f. *lycopersici*		> 250.0
Do *nicotianae*		> 250.0
Do *pisi*		> 250.0
Glomerella cingulata	3	0.8
Do	16	1.6
Do	18	3.1
Do	22	6.3
Do	28	62.5
Nigrospora sphaerica		0.2
Phoma lingam		0.4
Pythium sp.[c]		250.0
Sclerotinia fructicola	12	1.6
Do	118	0.4
Stemphylium sarcinaeforme		1.6
Venturia inaequalis	365–4	0.8[d]
Do	454–8	0.8[d]
Do	637–2	0.4[d]
Do	898–4	0.4[d]

[a] All fungi except *V. inaequalis* were incubated two days at 24° C. before data were taken; *V. inaequalis* was incubated two weeks at 16° C. *B. cereus* var. *mycoides* was incubated 24 hours at 37° C. before data were taken.
[b] The smallest amount of the antimycin preparation (dry weight) that completely inhibited growth.
[c] Streaked with a suspension of mycelial fragments.
[d] Results based on one trial.

of the antimycin preparation were used as controls[9]. Plates were streaked with the test organisms, incubated at a temperature suitable for the growth of these organisms, and noted for inhibition when growth became clearly visible on control plates.

A summary of the data is given in table 2. Entries are based on two or more trials, except as noted. The growth of those species that were not completely inhibited at the highest concentrations used was markedly re-

[9] The maximum ethanol content of test and control media was one per cent.

tarded. Amounts smaller than indicated in table 2 have been reported (11) to inhibit *Sclerotinia fructicola* and *Venturia inaequalis;* those data were obtained, however, with a preparation more purified than that used in the present studies.

The end points given in table 2 are for the conditions noted; under other conditions different end points were obtained. For example, if instead of fungus spores, small fragments of mycelium were used in seeding, much greater concentrations were needed to prevent growth. Incubation time was also important. The end point for *Nigrospora sphaerica* was found to be 0.2 μg. per ml. at the end of a two-day incubation period; at the end of ten days, however, the end point was 1.6 μg. per ml. Probably many other factors are important in this connection.

DISCUSSION

The present paper has reported studies on the production, partial purification, properties, and *in vitro* biological activity of an antibiotic substance produced by an unidentified species of *Streptomyces*. This material, like viridin (3) and glutinosin (5), appears to be more generally effective against fungi than bacteria. While most of the fungi dealt with in the present paper are phytopathogens, recent studies, as yet incomplete, indicate that certain fungi pathogenic to humans are also inhibited by the antibiotic.

A partially purified material, which represented a 47-fold increase in activity over the culture filtrate, had an inhibitory effect on all of the organisms tested that were antagonized in the presence of the growing antibiotic organism. There were, however, marked differences in the amounts of this material necessary to inhibit different species and even different isolates of a single species. The order of this sensitivity was in a general way parallel to that observed in the presence of the growing antibiotic organism.

SUMMARY

A species of *Streptomyces* was antagonistic on agar to all of 33 fungi tested. It was not antagonistic on agar to a number of bacteria, including several species commonly used in antibiotic assays. The antibiotic, which is tentatively named antimycin, was produced in shake flasks and by tank fermentation. It was partly purified by ethanol extraction of the precipitate formed when the culture filtrate was adjusted to pH 2.5. The active material was nondialyzable, heat labile, and soluble in a number of organic solvents and in water at pH 9.3. Water solutions at pH 9.3 became inactive in a few days; water suspensions at pH 2.5 and ethanol solutions appeared to be stable. In agar-streak tests, the partly purified material prevented or reduced the growth of *Bacillus cereus* var. *mycoides* and all of the 16 fungi tested. The most sensitive organism, *Nigrospora sphœerica*, was completely inhibited by 0.2 μg. per ml.; several test organisms were

not completely inhibited by 250 μg. per ml., the highest concentration employed.

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STUDIES ON THE BACTERIOPHAGE OF XANTHOMONAS PRUNI

H. H. THORNBERRY[1], A. EISENSTARK[2]
AND H. W. ANDERSON[1]

INTRODUCTION

Studies on the bacteriophage of *Xanthomonas pruni* (E. F. Smith) Starr and Burkholder, were undertaken to expand the knowledge on this bacterial virus in light of the techniques, equipment, and concepts that have become available since the isolation of the phage in 1927 (1). Since this bacteriophage is among the first bacterial viruses recognized for plant pathogenic bacteria, its properties, in addition to current interest, are of historical significance. The purpose of this paper is to give the results (12) and to discuss the utility and implication of the findings.

METHODS AND STUDIES

The bacteriophage, when originally isolated and concentrated in 1927, was preserved in sealed glass tubes. In May, 1947, after 20 years of storage in darkness at room temperature, the phage at 10^{-1} dilution lysed young cultures of *Xanthomonas pruni* upon its initial addition to the culture.

Increase of phage titer was accomplished by shallow-broth culture which permitted dense growth of the bacteria. Rectangular two-quart milk bottles containing 100 ml. of sterilized liquid culture medium[3] were placed on their broad side after inoculation and at 22°–24° C. for 24 hr. The broth within the bottles was approximately 0.5 cm. deep and presented an exposed surface of about 2 cm.² per ml. Sufficient solution of oxygen was secured by these conditions to permit dense growth of the bacterium throughout the medium. Lysis of such cultures yielded phage with a titer of 10^{-9} after Seitz filtration for sterilization and removal of bacterial fragments and sediments. Twenty-ml. aliquots of this phage filtrate were stored in 25-ml. glass serum bottles with rubber stoppers for further studies. The titer of the originally isolated phage was 10^{-5} when stored. It was not determined when revived. Its maximal increase by 12 serial passages through 24 hours broth cultures 3.5 cm deep (5 ml. of broth per 15×150 mm. tube) was 10^{-7}. With shallow-broth culture the titer was increased from 10^{-7} to 10^{-9} in one lysis procedure.

Specificity of the phage was evident from phage inoculations to cultures of isolates of *Xanthomonas pruni* and of other plant pathogenic bacterial species. All isolates of *X. pruni* from peaches, plums, and apricots located

in various regions of Illinois (vicinities of Belleville, Carbondale, Centralia, Olney, and Urbana) were lysed by the phage. Other species of bacteria tested (*Erwinia amylovora* (Burrill) Winslow et al, *X. campestris* var. *armoraciae* (McCulloch) Starr and Burkholder, and *X. lactucae-scariolae* (Thornberry and Anderson) Burkholder (See 11)), the last distinguishable from *X. pruni* only by pathogenicity, were not lysed. The lack of lysis in a culture of *E. amylovora* isolated from apricot twigs which is a host for lysable *X. pruni* indicates that the host in which the organism is parasitic either does not contribute to the specificity of lysis or such determinative characteristic is readily lost during multiplication and growth in the artificial medium used for isolation and perpetuation of the pathogen.

Electron microscopy[4] was employed to disclose the morphology of any
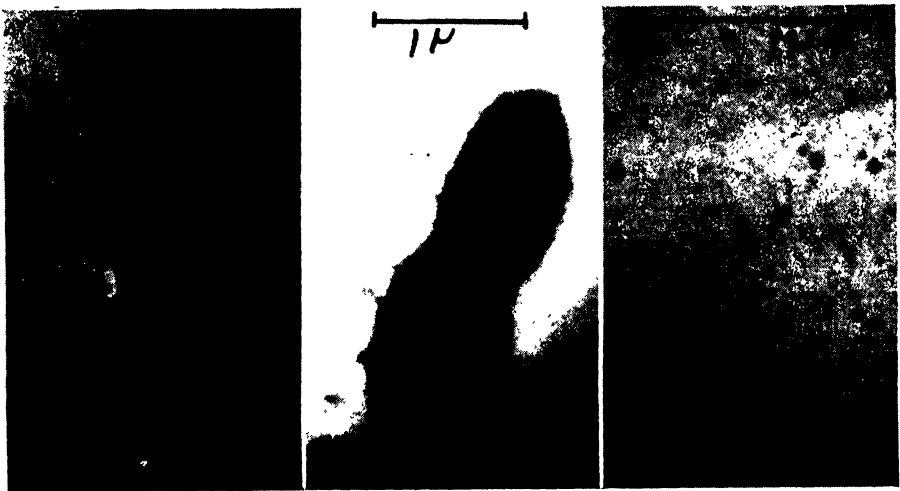
FIG. 1. Electron micrographs of *Xanthamonas pruni* bacteriophage particles (A) attached to a single cell, (B) attached to a constricted cell, (C) in suspension.

particles similar to those recognized as other bacteriophages and the adsorption of these particles by the host bacteria. The bacteriophage in a filrate of 10^{-9} titer was added to a suspension of growing cells of *Xanthomonas pruni* and then observed under the electron microscope (Type B, RCA). The bacteria were surrounded with small spherical particles, approximately 50 mμ (millimicrons) in diameter (Fig. 1). Electron micrographs of the phage filtrate revealed similar particles (Fig. 1). In very few cases, there was a suggestion that these particles possessed a "tail" or "handle" structure about 10 by 100 mμ. However, a "spherical" rather than a "tadpole" shape (particle with appendage) seemed to be characteristic of these particles. In this respect, the particles resembled the T3 and T7 bacteriophage of *Escherichia coli* (2), which are small spheres of 45mμ diameter. Also like the T3 and T7 bacteriophage, the particles in the

X. pruni phage filtrate seemed to have a low electron density, suggesting that they may be flat or that they may contain some water of hydration when in suspension and flatten upon drying.

Particles observed in the phage suspension were not always of uniform size. This may have been due to the possibility (a) that the larger particles were aggregates rather than individual bacteriophage units; (b) that the particles had occluded bacterial fragments; or (c) that the phage suspension was a mixture of several types of particles, such as the suspensions used by the earliest electron microscope workers in observing the attack of bacteriophages on *Escherichia coli* (2). By analogy with the electron micrographs of other bacteriophages, the spherical particles approxi-
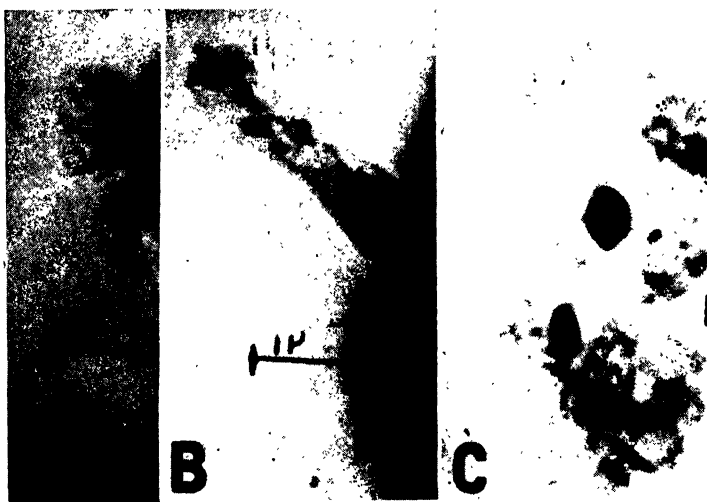
FIG. 2. Electron micrographs of lysed cells showing the region where rupture apparently occurred and some dense areas in the bacterial shell. (A) single cell, (B) double cell, (C) clump of cells.

mately 50 mμ in diameter and those with "tails" are assumed to be the bacteriophage. Evidence is lacking that these spherical particles or their "tails" are the actual infectious units.

Bacterial cells remaining after lysis (Fig. 2) show the place where rupture apparently occurred and some dense areas of the bacterial shell which may be adsorbed particles. Phage placed on established bacterial growth on agar slants lysed the area of the growth where streaked.

DISCUSSION

The aging of *Xanthomonas pruni* bacteriophage in suspension (lysed culture filtrate) for 20 years exceeds the 12-year aging period reported for *Bacterium stewarti* bacteriophage in sealed tubes at room temperature (9). However, this survival period of a bacterial virus is surpassed by the reported 52-year survival of a plant virus (tobacco mosaic virus), in dry tobacco-leaf tissues stored at room temperature (4).

The specificity of the *Xanthomonas pruni* bacteriophage offers a convenient phage-lysis technique for the identification of *X. pruni*. With other bacteriophages the technique has been successfully demonstrated for plant pathogens, *Erwinia amylovora* and two strains of *Aplanobacter stewarti* (7). Since *X. pruni* phage is stable at room temperature and apparently specific in its lysis, the phage-lysis technique is of considerable utility in the general routine identification of the bacterium and the diagnosis of plant diseases caused by this organism. Once the phage is prepared for storage the technique is simple and does not require expensive or complicated equipment or any special skill. For such use samples of 10^{-9} titer bacteriophage have been stored.

The size of the particles assumed to be *Xanthomonas pruni* bacteriophage is of the same order of magnitude as some other phages when determined by electron microscopy (2, 5). By ultrafiltration, however, the *X. pruni* phage has been reported to be 11 mμ in diameter (10). This estimated size was calculated from the smallest membrane pores (33.84 mμ diameter) through which the phage passed. The filtrate secured through these membranes did not cause lysis upon its initial addition to young cultures but required a subsequent transfer in a young culture before lysis occurred. At that time this was considered to mean that the phage was not in sufficient concentration in these filtrates to bring about visible lysis when originally added to a young culture. Lysis from original inoculum occurred with filtrates obtained through membranes with pore diameters as small as 68.32 mμ (10). If this is taken as the end point in filtration, the calculated size of the phage would be 53 mμ which agrees with the size of particles recognized by electron microscopy. The 11-mμ size approximates the smaller dimension of the phage "tails." The results from ultrafiltration and electron microscopy suggest (a) that the appendage is removable from the particle, (b) that the ultrafiltration through 33.84-mμ pores permitted only the appendages to pass, and (c) that these appendages after a period of association with the proliferating host cells then become the complete and specific bacteriophage. The suggestion that the appendage may contain the specificity portion of bacteriophage is further supported by the fact (a) that phages or their precursors require an association with a given organism through a series of transfers in order to become demonstrable (6, 8, 9), (b) that some particles assumed to be *X. pruni* bacteriophage in the electron micrographs, are without appendages, and (c) that specificity of a phage is related to the host on which it is propagated from its common origin (6, 8, 9). Furthermore, the orientation of adsorbed particles on bacterial cells appear to be with appendage toward the cell as is also suggested by some of the adsorbed particles in the electron micrographs of *Escherichia coli* bacteriophage (5) when enlargement prints are examined. However, drawn illustrations show the adsorbed orientation with the appendage away from the cell (3).

SUMMARY

Xanthomonas pruni bacteriophage remained viable for 20 years in sealed tubes at room temperature in darkness.

The bacteriophage titer was increased to 10^{-9}.

The bacteriophage is specific for *Xanthomonas pruni*.

The particles assumed to be bacteriophage are about 50 mμ in diameter.

Some particles appear to have an appendage about 10 by 100 mμ and resemble the T3 and T7 *Escherichia coli* bacteriophage particles.

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REPORT AND ABSTRACTS OF THE THIRTIETH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 30th annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of California, Berkeley, California, June 22-25, in conjunction with the meeting of the Pacific Division of the A.A.A.S. Approximately 100 persons attended the meetings. Arizona, British Columbia, California, Hawaii, Oregon, Utah, and Washington were represented. Thirty-five papers were presented on June 22 and 23. A field trip to the east and south of the Bay region on June 24 provided a survey of a wide variety of diseases. Four discussion sessions were held Friday morning, June 25. Topics were: "Control Measures for Plant Viruses," "Problems in the Identification and Classification of Fungi," "Recent Developments in Chemical Control of Plant Diseases," and "The Role of Plant Breeding in the Control of Plant Diseases." Presentation of six papers in a joint session with the American Society of Plant Physiologists and Botanical Society of America on "Biology of Fungi" concluded the program on Friday afternoon.

At the business session June 23 the following officers were elected for the calendar year 1949:

> *President:* George W. Fischer
> *Vice-president:* Wm. C. Snyder
> *Secretary-Treasurer:* George A. Zentmyer (2 year term)
> *Councilor:* L. C. Cochran (2 year term)

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Effects of amino-acridine derivatives on tissues of potato (Solanum tuberosum). ALPEN, E., JEAN DUFRENOY, and W. D. KUMLER. Discs punched out of the tubers of various genotypes of *Solanum tuberosum* were immersed in a shallow layer of solutions of amino acridine derivatives of concentrations ranging from 0.0004 M to 0.001 M. The relative "cytotoxicity" of the various compounds was evaluated in terms of the number of days or weeks during which the discs remained whole and sound, of the rate of formation of cicatrization layer at the cut surface, of the proportion of cells retaining the property of actively adsorbing vital dyes in their vacuolar solution, and last, of the rate of amylolysis and the fate of amyloplasts and mitochondria.

Susceptibility of some citrus species and other plants to the citrus-root nematode, Tylenchulus semipenetrans. BAINES, R. C., O. F. CLARKE, and W. P. BITTERS. In the field under conditions of general natural infestation of the citrus-root nematode (*Tylenchulus semipenetrans*) roots of the following were found to be infested moderately to severely: *Citrus aurantium* (29 varieties), *C. aurantifolia, C. celebica* var. *southwickii, C. grandis* (8 varieties), *C. hystrix, C. ichangensis, C. limon* (10 varieties), *C. medica, C. paradisi* (5 varieties), *C. reticulata* (6 varieties), *C. sinensis* (18 varieties), *C. tiawanica, Atalantia citroides, Fortunella* sp., *Microcitrus australasica, Microcitrus australasica* var. *sanguinea;* the following hybrids—*C. aurantifolia* × *F.* sp., *C. ichangensis* × *C. grandis, C. limon* × *C. paradisi, C. macroptera* × *C. reticulata, C. reticulata* × *C. grandis, C. reticulata* × *C. sinensis, C. sinensis* × *Poncirus trifoliata, C. tachibana* × *C. reticulata,* Citrange × *C. sinensis, P. trifoliata* × *C. limon, P. trifoliata* × *C. paradisi, P. trifoliata* × *C. sinensis* × *F.* sp. Roots of the following were free from this nematode: *Balsamocitrus dawei, Clausena lansium, Murraya paniculata* and *Severinia buxifolia.* Three of 20 *P. trifoliata* also were free from the citrus-root nematode. The resistant plants may be valuable in developing nematode-resistant rootstocks for citrus.

A leaf and twig blight of California laurel, Umbellularia Californica Nutt. BARRETT, J. T. A leaf and twig blight of the California laurel has been observed on the Berkeley campus for the past fifteen years. Many trees are slightly infected, others severely, and a few have been removed because of the disease. Infection by the pathogen, a species of *Macrophoma,* takes place mainly in the leaf tips from water drops carrying spores, and through the stomata which occur only on the dorsal side. From the leaf the fungus enters the twig which soon dies. If on a branch or limb, a canker may result and continue to develop two or more years. Tests have shown that infection may result within 48 hours after spores are placed on uninjured leaves and young shoots. Inoculation of young shoots of genera related to the laurel, such as *Persea gratissima, Persea india, Persea lingue, Cryptocarya mierri,* and camphor showed all to

be mildly susceptible. A number of unrelated forms subject to attack of Macrophomas were resistant to the laurel pathogen. All attempts to find in nature or to induce in culture a perfect stage have, to date, failed.

Some conditions contributing to the development and germination of oospores in the genus 'Phytophthora. BARRETT, J. T. In a previous report (Phytopath. 38: 2) the production of oospores by pairing species and strains of *Phytophthora* was reported by the writer. Similar results have been attained by pairing male and female strains of *Phytophthora drechsleri* with several isolants of *P. palmivora*, which demonstrate sexual compatibility between these two species and confirm some results of previous workers that two sex strains occur in the latter species. Oospores resulting from crossing the two sex strains of *P. drechsleri* have geen germinated, and 22 cultures from as many oospores have been established. No oospores have been found in any of these single cultures but when backcrossed with the two parents, 14 reacted with the male parent only, and six with the female only. In each case an abundance of oospores resulted. Oospores resulting from crossing two isolants of *P. infestans*, one from potato and one from tomato, with the female *P. infestans*, were also germinated, but only one culture from them has as yet been established. When backcrossed with the female *drechsleri* parent this culture produced an abundance of oospores, but none when mixed with the male strain.

Soil disinfestation in citrus orchards against armillaria root rot. BLISS, DONALD E. *Armillaria mellea* was found to a depth of 9 feet in sour-orange roots. Treatments with carbon disulfide were made in nearly undisturbed orchard soils containing artificially inoculated citrus root segments, 15 to 57 mm. diam. and 17 cm. long, planted at 1-foot intervals in vertical series 8 or 10 feet deep. Culture tests were made after 75 or 98 days. Charges of CS_2, applied at 18-inch, staggered intervals, killed armillaria in roots as follows: In coarse sandy loam near Azusa, California, 59 ml. at 1 foot killed from 1 to 7 feet, inclusive; 118 ml. at 1 foot killed from 1 to 8 feet; and 118 ml. at 6 feet killed from 3 to 10 feet. Near Rivera, in fine sandy loam underlaid with a clay-bearing substratum (5 to 6 feet) and sand, 44 ml. at 1 foot killed from 1 to 3 feet; and both 59 and 88 ml. at 1 foot killed from 1 to 5 feet. Surface charges failed to penetrate the clay substratum, deep charges being required. *Armillaria* remained viable in nontreated roots. *Trichoderma viride* developed in 54 to 100 per cent of cultures from treated roots, but in only 25 per cent from nontreated roots.

Exosporina branch blight of grapefruit in southern California. CALAVAN, E. C., and J. M. WALLACE. A branch blight of Marsh grapefruit was observed in November, 1945, in the Coachella and Imperial valleys of California. The withered branches contained spores of a toruloid fungus beneath the loosened periderm and frequently were associated with an anomalous form of trunk and branch gumming, with psorosis, or with both. Although confined principally to the smaller branches, blight lesions occasionally extended down primary branches to the trunk. The disease appeared to be more severe on weak trees than on healthy ones. Isolates of the fungus appeared nearly identical to isolates of *Exosporina fawcetti* Wilson from walnut. Ninety wound inoculations with pure cultures of *E. fawcetti* (from grapefruit) in April and July, 1947, on Marsh grapefruit trunks, branches, and twigs produced lesions in all cases. Forty-eight check wounds healed rapidly without infection. The fungus was easily recoverable from these inoculations as late as May, 1948.

Differences in susceptibility of lemon strains to dry bark. CALAVAN, E. C., and F. A. WHITE. Apparent differences in susceptibility of certain lemon strains to dry bark were observed in a few orchards in 1946, but the determination of resistant and susceptibile selections has been retarded by the general lack of detailed source records for most diseased plantings. Recent surveys in cooperation with Citrus Field Research, Inc., of Santa Barbara County, California, involving 15 nursery strains for a total of 3,917 trees in 8 orchards where careful records had been kept, revealed marked differences in the amounts of dry bark present in various strains. Six of the 15 strains were entirely free from dry bark, whereas the other 9 strains broke down in percentages ranging from 7 to 73 per cent. The 9 susceptible strains developed dry bark in all orchards where they were known to be planted. Tree susceptibility in the orchards studied (age 9 to 16 years) showed no important relationship to age, variety (Eureka and Lisbon), or rootstock.

Effects of various chemicals as growth-regulators. CARLO, P., JEAN DUFRENOY, and PETER SAH. Various compounds endowed with marked fungistatic activity and notably

Vitamin K₅ synthesized at the College of Pharmacy, U. C. Medical Center, San Francisco, have been demonstrated to show properties of growth-regulating hormones at concentrations ranging from 1 to 100 p.p.m. Their effect on the rooting of cuttings or on the germination of seeds was also studied in the presence of dithiobiuret, which, by itself has remarkable growth-regulating properties.

Mutation in bean rust uredospores in cold storage. DUNDAS, B. Uredospores of a number of forms of bean rust produced on detached bean leaves in Petri dishes collected during the summer of 1946 were subject to varying temperatures before being placed in storage at 0° F. in the fall of that year. When taken out in July, 1947, very few of the spores were viable, some collections giving no infection and the rest only a few pustules. Isolations from these pustules, tested on nine differential varieties, were in every case different from the form as stored, and in most cases different from previously described forms. Numerous isolates from a mixture of uredospores of 15 pure forms yielded only eight forms, six of which were different from any previously described. Since the unmixed uredospore collection of the other two forms yielded only mutant forms it is considered likely that these also may have been mutants, especially since both of these forms have come out as mutants from other pure forms. In all, nine mutant forms have been isolated. Mutation to the same form has occurred in several different forms and two or more mutants have been obtained from a single pure form. No consistent increase or decrease in virulence has been observed.

Disinfectant washes for the control of decay in apples and pears. ENGLISH, HARLEY. Various disinfectant washes were tested for the control of blue mold (*Penicillium expansum*) and bull's-eye rot (*Gloeosporium perennans*) of apples and gray mold rot (*Botrytis cinerea*) of pears, using punctured, inoculated fruit. Of the phenol derivatives tested, sodium chlororthophenylſ henate proved most effective whether used in a rinse or in an alkaline wash, reducing both blue and gray mold rots by 80 to 95 per cent. The thiocarbamates were ineffective against blue mold, but the soluble salts were highly effective against gray mold. Neither the phenol derivatives nor the thiocarbamates controlled bull's-eye rot. The quaternary ammonium compounds showed promise only in the control of bull's-eye rot. Alkyl dimethyl benzyl ammonium chloride was effective against the latter whether used in a final rinse or in an acid wash. With the exception of several mercury compounds, none of the other fungicides tested was so effective as sodium chlororthophenylphenate for the control of blue and gray mold rots. In semi-commercial tests with apples, sodium chlororthophenylphenate in a final rinse reduced decay 43 to 75 per cent. In similar tests with pears, the addition of this compound either to a final rinse or to the alkaline wash reduced decay 65 to 86 per cent.

The effect of particle size and solubility of sulfur in carbon disulfide upon its toxicity to fungi. FEICHTMEIR, E. F. The influence of allotropic form, particle size, and type of grinding upon toxicity of sulfur toward fungi has been investigated using particle size ranges of ±1 μ. These narrow size ranges were separated by means of liquid sedimentation. The results are itemized. 1. With sulfur particles 1–2 μ in diameter the concentrations of CS_2-insoluble and CS_2-soluble sulfur, respectively, in μg per ml. for 50 per cent reduction in germination or infection were as follows: for germination of conidia of *Sclerotinia fructicola*—3.9 and 13, for germination of conidia of *Erysiphe graminis hordeii*—0.6 and 2.1, for protection against *Uromyces appendiculatus*—30 and 80, and for protection against *E. polygoni*—1.9 and 12. 2. The LD 50 against conidia of *S. fructicola* for CS_2-soluble sulfur particles of 1μ, 3μ, 5μ, and 8μ radius decreased directly as the surface area per unit weight increased. 3. Agglomeration reduced fungicidal effectiveness. 4. Large particles remained toxic longer than small ones. 5. Micronized sulfur showed no greater innate toxicity than Raymond mill ground sulfur. In addition, an amorphous form (particles approximately 2μ in diameter) produced by evaporating an acetone solution of sulfur gave a high toxicity against conidia of *S. fructicola*—LD 50 of 0.13 μg per sq. cm.

Curly top of muskmelon. GIDDINGS, N. J.

A canker disease of figs. HANSEN, H. N. A canker disease of fig trees has been under investigation since late summer of 1947 when it was first observed in one of the highest producing orchards in California. The disease so far has been seen only on the variety White Adriatic and it appears to be confined to the older parts of trees since lesions have not been found on wood less than four years old. The younger cankers are rather inconspicuous and usually consist of a slight cracking of the outer bark. When scraping away the bark down to the cambium, one may find that a half-inch

crack on the surface often accompanies a six-inch canker at the cambium. Isolations from the margins of cankers consistently yielded a bacterial organism which when inoculated into the cortex of six-year-old wood of a fig tree gave rise to typical cankers. A similar disease occurs on fig trees in Italy where the causal organism is known as *Bacterium fici*.

Compatibility and sex in Hypomyces solani f. cucurbitae. HANSEN, H. N., and WILLIAM C. SNYDER. This heterothallic fungus occurs in four sex forms: hermaphrodites, females, males, and neuters. The compatibility group to which individuals of the above groups belong is determined by applying conidial suspensions of each of them to two tester strains, one known to be + and the other −. If the individual produces perithecia when mated with + it belongs in the − compatibility group and *vice versa*. The neuters, which are sexless and therefore unable to produce perithecia when mated with the + and − tester strains nevertheless give a positive reaction in, that a conidial suspension of a neuter will completely inhibit trichogyne formation in one of them and not in the other. Neuter + will inhibit trichogyne formation in tester − and *vice versa*. Thus the factors for heterothallism + and − are also present in sexless fungi.

Methods of inoculation of barley with the stripe disease, Helminthosporium gramineum. HOUSTON, BYRON R., and JOHN W. OSWALD. Numerous tests were conducted to determine a practical yet efficient method of inoculation of barley with the stripe disease fungus, *Helminthosporium gramineum*. Tests included spore suspensions applied to seed with and without vacuum, dried mycelial masses applied to the seed, and germination of seed in contact with growing cultures. Only the latter method resulted in consistently high percentages of infections. This method consisted of placing dry barley seed on the surface of a four-day Petri dish agar culture, inverting a similar culture over the seed, and allowing the seed to germinate. Employing this method, twelve time intervals of inoculation (constant 12° C.) ranging from 24 to 120 hours showed at 24 hours, 53 per cent infection; at 48 hours, 86; at 60 hours, 94; and all over 60 hours a percentage infection over 94. Seed dried for 10 days following inoculation resulted in no significant difference in percentage of disease except for the 24-hour interval. Inoculations carried to the same degree of seed germination at six temperatures ranging from 6° to 30° C. resulted in a high percentage of disease at all temperatures. Planting date and prevailing temperatures had little effect upon percentage of disease. Best infection occurred with this method at 12° C. for 96 hours.

A disease index for verticillium wilt of pepper. KENDRICK, J. B. JR., and JOHN T. MIDDLETON. Seedlings of *Capsicum frutescens* L. infected with *Verticillium albo-atrum* Reinke and Berth, have a characteristic stunting which may be classified and analyzed statistically. Stunting of inoculated plants is caused by reduced growth of internodes not fully expanded at the time of infection. A disease index is derived by comparing the individual length of an infected plant with the mean length of noninfected plants. Measurements are taken at the time of inoculation and four weeks later, for both inoculated and noninoculated plants. The difference represents the interim growth for four weeks of infection and normal expansion. The quotient computed by dividing the mean four-week growth of noninoculated plants by the same measurement of inoculated individual plants is the disease index. Relative severity is thus evident by the index, the greater the index the more severe the disease expression. This disease index is useful in a disease resistance breeding program where it is essential to preserve plants for subsequent hybridization and seed production. Confidence limits are established statistically for populations of noninoculated plants. Inoculated plants with an index within the confidence limits are regarded as highly resistant. Arbitrary classes may be established to include plants with different degrees of disease resistance.

Observations on the effect of 2,4-D on citrus fruit-stem dieback. KLOTZ, L. J., and W. S. STEWART. Dying of fruit stems occurs generally throughout California citrus orchards and causes important losses of crop in certain sections. Dying starts near the fruit button and extends a few inches to as much as 3 feet up the stem. The fruit then wilts or drops. Cause of the trouble is attributed to adverse climatic and soil factors acting to modify the physiology of the tree. During periods of water stress, gums may form and interfere with subsequent transport of water. The same stimuli may incite maturation of the abscission layer. Affected stems are invaded by fungi and bacteria which play an important secondary role in killing the stems after the initial injury. The chemical, 2,4-dichlorophenoxyacetic acid, applied in May and June, has been found to delay abscission and fruit drop, and to reduce fruit-stem dieback as much as 80 to 90 per cent.

Control of citrus brown rot, gummosis, and rind oil spotting by long exposures to low, moist heat. KLOTZ, L. J., and H. D. EVANS. The development of phytophthora brown rot in inoculated lemons, incubated 12 to 72 hours at 45°, 55° or 65° F., was stopped by an 18-hour exposure to air at 105° F., and 70 per cent to 96 per cent relative humidity. At 65° F. the fungus produced brown rot spots $\frac{1}{16}$ inch in diameter after 48 hours, and $\frac{1}{4}$ inch spots after 72 hours incubation, making the fruit unfit for use even though the heat treatment checked further development of the decay. All the other lemons used in the experiments had no blemishes. The treated fruit following 4 minutes' immersion in soda ash (1¾ per cent)–soap (½ per cent) solution at 120° F. or water at 120° F. did not develop rind oil spots. Budded lemon trees on sweet stock can tolerate a wet bulb temperature of 98° F. for 25 hours, an exposure lethal to the gummosis fungi.

Effects of combining fungicidal treatments and coating of seeds. LEACH, L. D. Tests were conducted in both pasteurized and Pythium infested soil to determine the effects of coating and incorporation of fungicides on emergence and infection of seedlings. In most cases, the coating of tomato seeds resulted in a slight delay of emergence, but did not reduce the number of seedlings. Coating alone reduced pre- and post-emergence infection, but the addition of fungicides such as Arasan (tetramethyl thiuram disulfide) and Phygon (2,3-dichloro-1,4-naphthoquinone) increased protection. The delay of emergence following coating of tomato seed previously treated with HgCl₂ or New Improved Ceresan (ethyl mercury phosphate) dip, was greater than the additive delays from coating and treating separately. The inclusion of organic mercury compounds in the coating, resulted in reduced and retarded emergence. With processed sugar beet seed a neutral reaction of the coating was most favorable for emergence, but an acid reaction enhanced protection. Coating alone provided about half as much protection against Pythium infection as Arasan or Phygon in the coating material. Coating onion seed with or without Arasan at a dosage of 5 per cent of the seed weight, neither reduced nor delayed emergence. However, emergence was delayed by 50 per cent when Arasan at 75 per cent of the seed weight, was included in the coating for smut control.

Attraction of the vascular bundles for dodder haustoria in healthy and curly-top infected beet petioles. LACKEY, CHARLES F. Comparisons are made between dodder haustoria and beet leafhopper stylets in their ability to go directly to the vascular bundles of the beet petiole. Both go directly to the bundles in the majority of cases and can turn to a bundle when continuation in the original direction would have passed it. Neither dodder nor leafhopper will penetrate very deeply on the upper side of the petiole in tissue well away from the vascular bundles. Neither one will live long without vascular contact. Bennett (Jour. Agr. Res. 48: 665–701) showed that in 46 per cent of the cases the leafhopper stylets went directly to the bundles, 46 per cent started toward bundles but did not go deeply enough, and only 8 per cent were on the concave side of the petiole in tissue well away from the bundles. In healthy beet petioles the dodder went directly to the bundles in 97 per cent of the cases and missed in only 3 per cent. These data suggest a similar attraction of the vascular bundles for both the leafhoppers and dodder. In curly-top infected petioles only 53 per cent of the haustoria went directly to the bundles and in 47 per cent they missed the bundles or did not go deep enough. No studies regarding the effect of curly top infection on the feeding of the beet leafhopper have been reported.

Studies on the chemotherapy of potato virus diseases. LOCKE, SETH B. Treatment of a hill of leafroll-infected Netted Gem potatoes with 0.2 per cent 2,4-dichlorophenoxyacetic acid resulted in the complete masking of symptoms in the new growth produced after treatment, and also in the first vegetative generation of plants grown from the tubers of the treated plant. Grafts made at intervals from the first vegetative generation progeny of the treated plant to healthy plants showed no transmission at first, and later, increasing proportions of transmission up to 100 per cent. Symptom development was slower in the earlier transmissions and more rapid in the later ones relative to rate of symptom development following transmission from non-treated leafroll infected plants. These results are interpreted as indicating a reduction in amount of active virus in the treated plants and not merely masking of symptoms. Reinoculation of first generation, vegetative progeny of the treated plant by grafting with scions from non-treated leafroll plants failed to induce symptom development. This may indicate that amounts of the active chemical sufficient to inhibit virus multiplication were present in the plants at the time of reinoculation.

Pythium wilt of Phaseolus, Pisum, and Vigna. MIDDLETON, JOHN T., and W. C. SNYDER. Pythium wilt of bean, pea, and cowpea is characterized by a light brown, watersoaked discoloration of the stem, usually ⅛ to ¼ inch wide, originating at the ground level and progressing upwards 6 to 12 inches, followed shortly by a conspicuous unilateral wilting of the plant. Susceptible hosts are most subject to infection with the advent of flower production. The disease is caused by several species of *Pythium: P. aphanidermatum, P. debaryanum, P. irregulare, P. oligandrum, P. splendens,* and *P. ultimum.* Varieties of *Phaseolus vulgaris* found naturally infected include: Kentucky Wonder, Red Kidney, Small White, Standard Pink, Small White Lima, and Ventura Lima. Varieties of *Pisum sativum* found naturally infected are: Alderman, Little Marvel, and Progress. Several varieties of *Vigna sinensis* have been found naturally infected. In California the disease is encountered wherever susceptible hosts are grown, but perhaps is more commonly noted in the Salinas and lower San Joaquin valleys. Although several *Pythium* spp. are concerned, *P. ultimum* is generally responsible for causing the disease in coastal areas, while both *P. ultimum* and *P. aphanidermatum* cause the malady in the interior.

A late-breaking virus disease of potatoes. MILBRATH, J. A., and W. H. ENGLISH. An unusual graft-transmitted virus disease of potatoes has been found in Oregon. Plants grow normally until late in the season and then they become off-color, the stems stand erect, aerial tubers develop in nearly every leaf aril, and the leaves became yellowed and rolled. Plants affected with acute symptoms remain small, yellowed, and have thickened nodes and aerial tubers. Outstanding points which differentiate this disease are as follows: it is tuber perpetuated for at least three generations; normal-appearing plants from infected tubers develop symptoms late in the season as plants reach full size; tubers are reduced in size, normal in number, and without internal symptoms; tubers from an infected hill may produce normal plants, weak plants, and plants with severe or mild symptoms; a single tuber cut in several pieces may produce plants which also show this variation in symptom development. This combination of symptom expressions does not fit any other virus disease associated with potatoes.

Arsenite injury to grape canes through leaf scars. NELSON, K. E., and WM. B. HEWITT. Sodium arsenite applied as a dormant spray to the canes, arms, and trunks of Thompson seedless vines (*Vitis vinifera* L.), on dry soil plots, caused appreciable injury as evidenced by bud killing and sharply defined necrotic areas in the xylem tissue below the leaf scar. Vines in a wet soil plot had only minor xylem injury with little bud killing. Vines sprayed only on the arms and trunks had considerably less injury, it being confined chiefly to the buds at the bases of the canes. Histological study showed that the leaf scar periderm was formed before leaf fall. Most of the vessels of the leaf traces in scars collected in December after leaf fall were open with no covering over the broken ends and little to no wound gum within the vessels. Some of the leaf scar traces collected in January were partially plugged and the remainder entirely plugged with gum. By February, plugging of the leaf scar traces was complete. A study of damaged buds indicated the sodium arsenite penetrated to the xylem tissue through the vessels of the leaf trace. From there, necrosis extended downward as far as four inches below the leaf scar and also upward into the bud.

The influence of fungicidal dusts upon the rooting of cypress and tamarix cuttings. NEWTON, WM. In the propagation of Cypress and Tamarix by cuttings, the loss of cuttings is greatly diminished by the simple practice of dipping the bases of the cuttings into dry Fermate (ferric dimethyl dithiocarbamate). This practice is more effective in the promoting of rooting and survival than a one-hour immersion in a solution of naphthalene acetic acid which contains 10 p.p.m. The inclusion of a trace of naphthalene acetic acid with Fermate and other fungicides had little significant effect upon the cuttings.

Leaf roll, net necrosis, and stem end browning in Netted Gem potatoes in California. OSWALD, JOHN W., and JAMES B. KENDRICK. Leaf roll is very serious in Netted Gem potatoes because current season infection markedly reduces yield and causes net necrosis in the tubers. Reduction in yield is directly proportional to the severity of plant symptoms. In addition to net necrosis, Netted Gem potatoes develop stem end browning. Tuber indexing showed net necrosis was definitely associated with leaf roll, whereas, stem end browning was not. Tubers from 90-day-old plants had only a trace of net necrosis, but after six weeks' cellar storage (about 45° F.) and cold storage at 36° F., showed 23 and 5 per cent respectively. Mature tubers harvested after killing frost, showed 40–50 per cent net necrosis and 10–15 per cent stem end browning with no subsequent increase

in either disease in either cellar or cold storage. Tubers from 90-day-old plants showed no stem end browning and subsequently developed very little in either type of storage, indicating the conditions bringing this about occurred between early and normal harvest. Rolling, removing, and killing plants before and after frost had little effect on the development of either type of browning. Stem end browning is definitely more severe on muck soils (39.3 per cent) than on sandy soils (4.6 per cent).

Sieve-tube necrosis, an early symptom of collapsing and declining lemon trees. SCHNEIDER, HENRY. Forty-two apparently healthy 14-year-old lemon trees budded on grapefruit stock exhibited necrosis of part or all of the sieve tubes just above the bud union in May, 1947. At ¼ inch and 6 inches above the union, necrosis was less extensive or absent, and none was observed below the union. The phloem of scaffold branches and twigs was usually normal. Starch, which was abundant in the wood of the roots of most of the trees in May, 1947, subsequently disappeared in most of them. By May, 1948, many of the trees exhibited yellowing of the leaves and partial defoliation; the fruit ripened prematurely and was of small size; and the more severely affected trees had failed to make a growth flush. The results indicate that necrosis of sieve tubes is an early symptom which causes starvation of the roots, which usually causes top symptoms similar to those of lemon decline, and occasionally causes a collapse of trees. In six other orchards on grapefruit stock and in one on sweet orange stock, observed over a shorter time, similar symptoms have been observed; but in two of the orchards only a few trees were affected. New phloem was present in trees that had collapsed or declined and then recovered.

Inheritance of spotted-wilt resistance in tomato. SMITH, PAUL G., and M. W. GARDNER. A high level of resistance to spotted-wilt in tomato has been demonstrated in the Red Currant tomato (*Lycopersicon pimpinellifolium*) under conditions of field infection, but it is susceptible when artificially inoculated. German Sugar, a variety of *L. esculentum*, is moderately resistant to field infection. Hybrids with Red Currant and the cultivated tomato have yielded lines with an intermediate level of resistance. These remain comparatively free of spotted-wilt under moderate epidemic conditions, but are badly diseased under severe epidemic conditions. The Red Currant level of resistance has not been recovered in any of its progeny, and no evidence of simple Mendelian inheritance of resistance has been observed. Hybrids of German Sugar and the commercial varieties yield F_1 and F_2 populations intermediate in resistance. In back crosses of the F_1 to the resistant parent, the German Sugar level of resistance was not recovered. The back cross of the F_1 to the susceptible parent yielded a level of resistance intermediate between them. Again no evidence of Mendelian segregation was observed. The reaction of the resistant and partially resistant varieties varies markedly in different years and localities, the causes of which are not clear.

Diseases of Guar (Cyamopsis psoraloides). STREETS, R. B. Guar has been grown in the Southwest in recent years as a summer green-manure crop and for the monogalactan gum of the seeds. No severe losses from plant diseases have occurred in Arizona or elsewhere, but 14 diseases and injuries, some more conspicuous than injurious, have been recorded. The branched Mesa variety is highly resistant to two diseases, root-knot caused by *Heterodera marioni*, and root rot caused by *Phymatotrichum omnivorum*. The unbranched, early-maturing Texsel variety is moderately susceptible to root rot, but resistant to Sclerotium rot. Some losses occur from Fusarium and Rhizoctonia root rots, and *Sclerotium rolfsii* is injurious in wet soils. Mosaic caused losses of seed yield up to 50 per cent in 1943 but has not reappeared. Alternaria leaf spot is the only common foliage disease and may cause more or less defoliation in years of greater than normal rainfall and humidity. Many plants of unbranched varieties are stunted and finally die from an injury, of unknown cause, to the growing point when the plants are 6 to 18 inches high. Two diseases reported elsewhere have not been observed in Arizona: lethal virus (Oklahoma) and powdery mildew (India). Five minor diseases not mentioned above have been observed.

Profitable use of root-rot-infested irrigated land. STREETS, R. B. Cost analyses have been made on seven rotations with cotton or flax as the principal cash crop in comparison with continuous cotton on root-rot-infested irrigated land for the years 1944–47, inclusive. To the normal cost of growing the crops was added the expense for materials and labor used in different treatments and rotations. The average per acre profits for the replicated plots for 1946 and 1947 were computed, as the effects of treatment and rotation are cumulative. Root rot, caused by *Phymatotrichum omnivorum*, was reduced in varying amounts, but not eliminated from the plots, but the net returns per acre were increased. The rotations in ranking order of profit (1946–47 averaged)

were as follows: (1) barley, guar for seed, sulphur and nitrogen, cotton (two-year rotation) ; (2) winter legume for green manure, cotton; (3) manure in furrows under cotton rows; (4) early guar for seed, flax; (5) guar for green manure, flax; (6) manure in furrows plus sulphur and nitrogen; (7) sesbania for green manure, flax; and (8) continuous cotton. Rotations 3 and 6 require too much labor and expense for materials to be useful in general practice.

The effect of temperature on the taxonomic characters of Verticillium albo-atrum Rke. et Bert. WILHELM, STEPHEN. A temperature difference of 3°–6° C. within the growing range (10°–31° C.) of *Verticillium albo-atrum* Rke. et Bert. may produce in this fungus marked differences in cultural appearance and in morphological characters, particularly of the resting stages. Colonies grow at low temperatures (10°–22° C.) or during the winter at Berkeley, California, are jet black and growth consists almost entirely of thick microsclerotial crusts. At higher temperatures (25°–31° C.) or during the summer at Berkeley, colonies are creamy white and have only sparse development of microsclerotia. Thus the resting structures of this fungus, in particular the microsclerotia, and colony appearance are not reliable characters upon which to separate species. Colonies grown at low temperatures answer to the concept of *V. Dahliae* Kleb. as interpreted by Van der Meer and Keyworth and to the *Niger* group of *V. Dahliae* as described by Berkeley. Only the wild type or conidial constituent of *V. albo-atrum* respond as described to temperature, the stable white mycelial variants being unaffected.

A dwarfing virus disease of bramble fruits. WILHELM, STEPHEN, H. E. THOMAS, and D. D. JENSEN. A graft transmissible disease of bramble fruits, serious in California mainly in the Logan variety, is proven to be of virus nature. Diseased Logans are characterized by marked dwarfing of cane growth, weak development of fruit laterals, downward cupping and yellow-bronze coloring of the leaves, premature reddening in the fall, and precocious development of basal buds during plants, late in the season, a bunchy appearance. Plants are slow to start in the spring, and when diseased a year or more are unproductive. The varieties and species of blackberry related genetically to the Logan, i.e., Mammoth, Cory's Thornless, and Phenomenal, and the Pacific Coast trailing blackberry (*Rubus ursinus* Cham. & Schlecht.) are readily graft inoculated and all but the last affected severely. Less severe symptoms are produced in the varieties Boysen, Young, and Nectar. In these varieties the disease has not been identified in the field. Preliminary tests with raspberry indicate that it may act as a symptomless carrier. The disease was produced in Logan when inarched to the wild Pacific Coast trailing blackberry in 5 out of 35 trials, indicating that this wild blackberry harbors the virus.

Relation of temperature and moisture to the effectiveness of sodium pentachlorophenate as an eradicant spray against Sclerotinia laxa. WILSON, E. E. Measurable eradicative action by a sodium pentachlorophenate spray preparation used against sporodochium-borne conidia of *Sclerotinia laxa* on almond twigs, was not detected during the short period required for the spray to dry on the twig, or during dry weather. Continuous wetting of the twigs was more favorable to eradicative action than intermittant wetting; the percentage mortality of conidia on sprayed twigs increased progressively as the period of moisture continued for eight days. Heavy rains not only checked destruction of conidia by the spray, but allowed new sporodochia to develop. Mortality of sporodochium-borne conidia receiving the sodium pentachlorophenate spray rose with successive increases of temperature between 7° and 30° C. Apparently this rise in mortality was occasioned by an increased eradicative action of the spray and not by high temperature since exposure of conidia to 24° and 30° C. did not materially affect their subsequent germinability. In the field such high temperatures did not occur, the range being from about 5° to 13° C. Within this range, development of sporodochia and the consequent production of conidia on untreated twigs increased rapidly with increased temperature. In a similar manner temperature affected the development of sporodochia on treated twigs that had been exposed to a considerable amount of rain after treatment with sodium pentachlorophenate.

Apricot jacket rot. YARWOOD, C. E. This disease, also called green rot, calyx rot, and blossom rot, is caused by *Sclerotinia sclerotiorum, Botrytis cinerea, Monilinia laxa,* and *Monilinia fructicola,* the fungi probably being of importance in this order. Infection starts in the senescent calyx, but may invade the enclosed or contiguous young fruit and cause it to rot and then drop. One or the other of the first two fungi have been regularly present in normal almond and apricot blossoms in all localities explored from 1943 to 1948 but have caused loss only during prolonged periods of wet weather. Fruit rot by the three genera can be distinguished by the color of the rot (being darkest with *Monilinia* and palest with *Sclerotinia*) and by the characteristic aerial mycelium or sporo-

phores produced after incubating specimens in a moist chamber. In Berkeley the *Botrytis* has predominated, and jacket rot caused by *Sclerotinia* has not been found with certainty, while in the Winters and Hollister areas the *Sclerotinia* has predominated. Jacket rot caused by *Sclerotinia* or *Botrytis* and the twig phase of brown rot caused by *Monilinia laxa* have been controlled by an application of 0.2 per cent Fermate (ferric dimethyl dithiocarbamate) plus spreader at full bloom.

Germ tube growth of some obligate parasites on agar media. YARWOOD, C. E. On washed non-nutrient agar at 22° C. the sporangia of *Peronospora destructor* produced unbranched germ tubes about 500μ long and then ceased growth. With the addition of 2 per cent sucrose, 0.05 per cent glycine, 0.05 per cent calcium glycerophosphate, 0.02 per cent dipotassium phosphate, 0.01 per cent histidine, 0.004 per cent glutathione, and 0.001 per cent disodium alpha tocopherol phosphate to the agar, branched germ tubes averaging about 2600μ with a maximum of 7000μ have resulted in 4 days. Differences in growth on controls and on test media have been greatest at 25° C., though the optimum for the organism is about 16° C. On non-nutrient agar at 16° C., germ tubes of the conidia of *Erysiphe graminis* from barley grew to about 44μ in 2 days, while on agar to which sucrose, Tween 60 (a polyoxyalkaline derivative of sorbitan monostearate), and disodium alpha tocopherol phosphate had been added the germ tubes averaged about 280μ with a maximum of 650μ. On non-nutrient agar at 20° C. the germ tubes of thinly-seeded uredospores of *Uromyces phaseoli* from bean averaged 390μ, while on agar to which sucrose, potassium permanganate, disodium alpha tocopherol phosphate, and glutamic acid had been added the germ tubes averaged about 1000μ.

Western X-disease of Montmorency cherry and its relation to buckskin of sweet cherry. ZELLER, S. M., and J. A. MILBRATH. Montmorency cherry trees infected with "little cherry" bear small green fruits that ripen late. The disease transmitted to sweet cherry causes buckskin disease and transmitted to peach trees causes Western X-disease. A table of 54 cross transmissions of Western X-disease of peach, red-leafed chokecherry disease, and the "little cherry" found in Montmorency cherry and several sweet cherry varieties in Wasco county, Oregon, indicates that all of these diseases are caused by the same virus, or that the infected trees contain the Western X-disease virus. The symptoms of this disease of sweet cherry look the same as those of the buckskin disease of sweet cherry in Green Valley, California. On the other hand, the disease in Oregon has not been demonstrated to react to Mazzard and Mahaleb rootstocks as reported by Rawlins and Parker for the buckskin disease of cherry in California.

Zinc Dimethyldithiocarbamate (Zerlate or Karbam White), A Promising Fungicide for Pecan Scab Control.—Home-made Bordeaux mixture, even after more than 30 years of investigations, has been the cheapest and most satisfactory fungicide for the control of pecan scab (*Cladosporium effusum* (Wint.) Demaree), a disease of major importance in the pecan industry. In 1946, tests[1] made on the Moore variety indicated that 3 lb. Copper A or Fermate per 100 gal. would control this disease, under conditions of moderate infection, as well as would low-lime Bordeaux mixture. From tests made at the U. S. Pecan Field Station, Robson, La., in 1946, Ralph H. Sharpe reports[2] that zinc dimethyldithiocarbamate (Zerlate) controlled pecan scab on the Schley and Pabst varieties, with a smaller build-up in the black pecan aphis (*Melanocallis caryaefoliae* (Davis)) population, as well as did low-lime Bordeaux mixture.

In 1947, tests using 2–100 Zerlate in comparison with Bordeaux mixture, each of them with and without Dupont spreader-sticker, were made by the writer to control scab on the Schley variety at Albany, Ga. The spray materials were applied as follows:

First application: When the first leaves formed were half grown and before pollination, 2–100 Zerlate or 4–1–100 Bordeaux mixture was applied.

Second application: An application of 2–100 Zerlate or 6–2–100 Bordeaux mixture was made soon after pollination was complete, or when the tips of the small nuts had turned brown.

Third and fourth applications: The third spray application was made about 3 to 4 weeks after the second, and the fourth about 3 to 4 weeks after the third, 2–100 Zerlate or 6–2–100 Bordeaux mixture being used for each.

The results of these spray tests are given in table 1.

TABLE 1.—*A comparison of one year's spraying results with Zerlate and with Bordeaux mixture to control pecan scab. Schley variety planted 10 trees per acre. Albany, Georgia, 1947*

Treatment	No. of trees per test	Yield in lb. per tree	No. nuts per lb.	Percentage of kernel in the nuts
4 applications, Zerlate 2–100	9	34	55	63
4 applications, Zerlate 2–100 plus 1 qt. Dupont spreader sticker	9	30	57	61
1 application 4–1–100 Bordeaux mixture followed by 3 applications 6–2–100 Bordeaux mixture	9	40	60	60
1 application 4–1–100 Bordeaux mixture followed by 3 applications of 6–2–100 Bordeaux mixture plus 1 qt. Dupont spreader-sticker	9	32	63	57
Check, no spray	9	1	88	51

[1] Cole, John R. A comparison of home-made Bordeaux mixture with other fungicides for control of scab on the Schley and Moore varieties of pecans. Phytopath. **38**: 106–109. 1948.

[2] Correspondence with Ralph H. Sharpe, formerly assistant pomologist, U. S. Pecan Field Station, Robson, Louisiana; also personal observation by the writer.

Both Bordeaux mixture and Zerlate controlled the scab disease (Fig. 1). Although the trees sprayed with Bordeaux mixture produced the greatest number of pounds of nuts, the Zerlate-sprayed trees produced the heaviest individual nuts, as well as the highest percentage of kernel in the nuts. This would indicate that the Bordeaux-sprayed trees had a heavier set of nuts to begin with than those that were sprayed with Zerlate, since there was no evidence that early applications of the Zerlate spray were injurious to the pistillate flowers. All treatments gave outstandingly higher yields of nuts per tree as well as higher percentages of kernel in the nuts than were obtained from the unsprayed trees. Likewise, the yield of nuts per tree was reduced and the nuts were somewhat lighter in the treatments in

FIG. 1. Clusters of Schley pecan nuts. A. Received 4 applications of 2-100 Zerlate and are free of scab and of No. 1 quality. B. Received one prepollination spray of 4-1-100 Bordeaux mixture and 3 applications of 6-2-100 Bordeaux mixture. Free of scab and of No. 1 quality. C. Received no spray. Scab has made these nuts unmarketable. All photographed in October, 1947.

which the spreader-sticker was used, which indicates that this material may have been detrimental.

The foliage was a healthier green on the Zerlate-sprayed trees during the late summer and early fall than on either the Bordeaux-sprayed trees or the unsprayed checks. This color may have been caused by the absorption of zinc which is an ingredient of Zerlate, although the trees have not shown any visible signs of rosette, a zinc deficiency disease.

The infestation of black aphis was not a serious factor in this spray test, even on the Bordeaux-sprayed trees. There were fewer aphids on trees that were sprayed with Zerlate; however, than on those that were sprayed with Bordeaux mixture. There was a greater build-up of the black aphis population on both the Bordeaux-sprayed and the Zerlate-sprayed trees than there was on the unsprayed checks.—JOHN R. COLE, U. S. Department of Agriculture, Pecan Field Laboratory, Albany, Georgia.

Sori of Urocystis gladioli on Gladiolus Corm Scales.—Previously published illustrations of the smut disease of gladiolus, *Urocystis gladioli* W. G. Smith, are confined to drawings and a photograph of the spore balls. This

name has been cited so generally as *Urocystis gladioli* (Req.) W. G. Smith that attention should be called to Mason's explanation[1] that the correct form is *Urocystis gladioli* W. G. Smith. Ramsbottom[2] accepts Mason's citation, and Linder[3] cites it in the same manner. Linder also offers evidence that Requien's fungus, *Uredo gladioli*, is a synonym of *Puccinia gladioli* Cast.
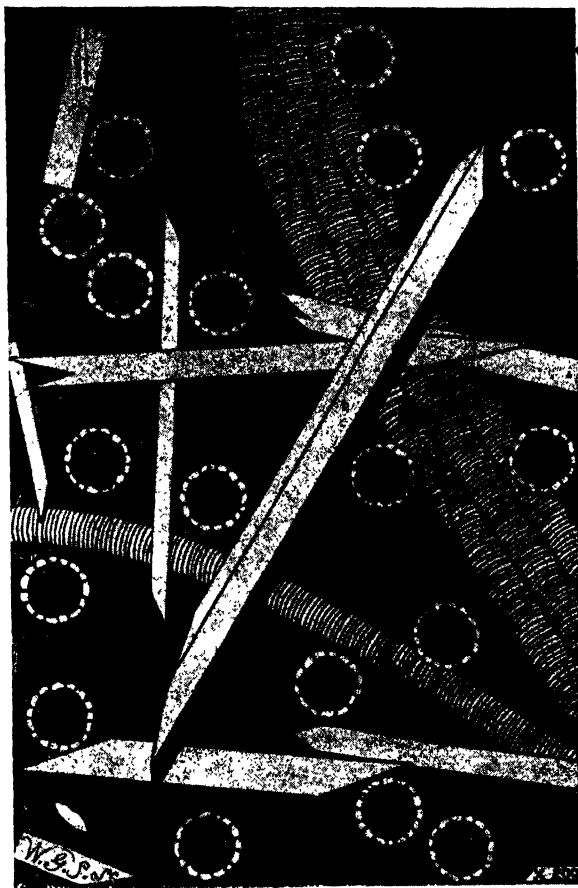
FIG. 1. Fragment of diseased corm showing decomposed cells, spiral vessels, crystals, and compound fungus spores, *Urocystis gladioli*, × 200. (After Smith[4, 5].)

The first illustration of *Urocystis gladioli* appeared in 1876 in the Gardener's Chronicle[4] with the original description of the species. Similar figures were also published by Smith[5] in the Monthly Microscopical Journal in the same year. Figures of the spore balls by Cooke[6] were later published

[1] Mason, E. W.   New species and old.   Trans. Brit. Mycol. Soc. 25: 433–434. 1942.
[2] Ramsbottom, J.   Conclusions and prospects.   Trans. Brit. Mycol. Soc. 25: 436–439. 1942.
[3] Linder, David H.   Note.   Mycologia 34: 398–399. 1942.
[4] Smith, W. G.   The gladiolus disease.   Gard. Chron. (n.s.) 6: 420–422, 2 figs. 1876.
[5] Smith, W. G.   The gladiolus disease.   Monthly Microsc. Jour. 16: 304–311, pl. 163, 164 a, b. 1876.
[6] Cooke, M. C.   Pests of the flower garden.   Jour. Roy. Hort. Soc. 27: 369–406, pl. 5, fig. 94. 1902.

in the Journal of the Royal Horticultural Society, and more recently a photograph by Hotson appeared in Mycologia.[7] These papers do not include an illustration of sori on the host, although Smith's figure 84, a
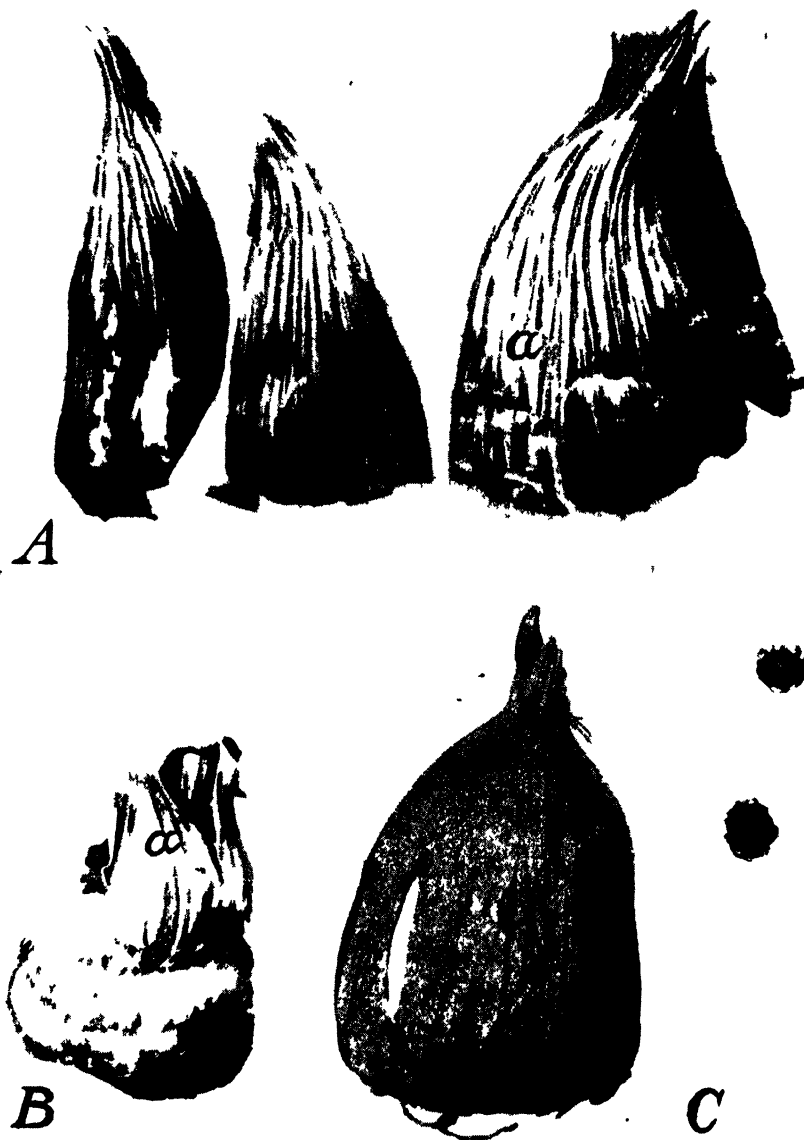
FIG. 2. *A.* Gladiolus corm scales bearing sori of *Urocystis gladioli,* × 2 1/2. *B.* Two gladiolus corms bearing smut sori, × 2. *C.* Spore balls of *U. gladioli,* × 580.

photograph of which appears here as figure 1, shows scattered spore balls on the host tissue.

[7] Hotson, H. H. The morphological distinction between *Urocystis gladioli* and *Papulaspora gladioli.* Mycologia 34: 52–58, fig. 1, 2. 1942.

Smith stated that there was a difference of opinion as to the injury caused by this smut, but van Poeteren[8] reported that in 1924 it was the cause of a serious disease of gladiolus in The Netherlands. It seems desirable, therefore, that American horticulturists and plant pathologists should become acquainted with the appearance of this smut in order that an introduction of the disease into the United States might be promptly recognized.

The photographs in figure 2 are of infected corms of gladiolus found in a shipment from Hillegom, Holland, at the Bureau of Entomology and Plant Quarantine inspection house at Hoboken, New Jersey, by P. E. Grayson on April 15, 1947.

The unbroken sori are roughly oval with the longer axis parallel with the nerves of the scale except when several sori become confluent (Fig. 2, A and B). The surface is roughened in parallel ridges which appear to be metamorphosed scale nerves. The grayish, lead color of the sori is little different from that of the healthy scale. Several sori in the figures appear too dark, but others are shown correctly (Fig. 2, a in A and B). The sori are usually raised slightly above the surface of the scale as low blisters. Those found on this material measure 4.5–6.5 by 1–5 mm., and are about 0.5 mm. thick. Two spore balls and a part of a third are shown in figure 2, C.

Grateful acknowledgment is made to John A. Stevenson, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, for advice in regard to the taxonomy of this fungus; to W. H. Wheeler, of the Bureau of Entomology and Plant Quarantine, for the photograph of Smith's figure and for assistance in obtaining bibliographical material; and to the Gardener's Chronicle Limited for permission to reproduce Smith's figure.—DONALD P. LIMBER, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Hoboken, New Jersey.

Cases of Scab on Violet and Pansy in Maryland.[1]—The extreme destructiveness of violet scab (*Sphaceloma violae* Jenkins) on cultivated and wild violets has become of greater moment as more and more instances of harm or loss from its attack have become known.[2] Transfer of affected plants

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[8] Poeteren, N. van. Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het Jaar 1924. Verslag. en Meded. Plantenziektenkund. Dienst Wageningen No. 41, 62 pp. 1925.

[1] Scientific Article No. A 197. Contribution No. 2111 of the Maryland Agricultural Experiment Station.

This is an elaboration of our unpublished note "A case of violet scab on pansy," presented (by Jehle) at the meeting of the Potomac Division, American Phytopathological Society, Bureau of Plant Industry Station, Beltsville, Maryland, on Feb. 11, 1948. Thanks are extended to Dr. W. F. Jeffers and to Mr. R. L. Taylor who made the photographs as acknowledged in figure 1.

[2] Noble, R. J. Notes on plant diseases recorded in New South Wales for the year ending 30th June, 1938. Internat. Jour. Plant Protect. 13: 25M–26M. 1939.

Smith, C. E., and A. G. Plakidas. Plant pests and problems. Home gardening for the South 6: 491. 1946.

Thaxter, K. Scab in a Connecticut violet collection with notes of varietal reaction. U. S. Dept. Agr., Plant Dis. Reptr. 29: 410–411. 1945.

Whetzel, H. H. The scab disease of violets. U. S. Dept. Agr., Plant Dis. Reptr. 28: 769–770. 1944.

without knowledge that they were diseased doubtless has been an important factor in spreading the pathogen from locality to locality. Fortunately the disease is still absent in many places within its reported range in the United States.[3, 4] In the region of the District of Columbia, however, whence comes the earliest available record (1897–1899),[3] it is more or less widely distributed.[3, 5]

In summarizing the distribution of the disease in Maryland, Walker et al.[6] referred to an occurrence in 1936 on cultivated violets in a small commercial greenhouse in Maryland. This had come to the attention of Dr. Mark Woods, then of the University of Maryland, soon after the original publication by Massey and Jenkins,[7] on the disease. Because of this, Dr. Woods requested that Jenkins verify his diagnosis.

Further data relative to this case of violet scab are as follows: In autumn, 1935, plants of the varieties "Freys Fragrant" and "Princess of Wales" were purchased from a northern violet grower. They were full grown and diseased when received. When advice was sought several months later, most of the plants were practically ruined. A few of the less severely infected ones were removed from the greenhouse and planted outside.

These details were furnished recently by the grower[8] who again requested advice of the University of Maryland on a disease that was ravaging her pansy seedlings in autumn, 1947. Jehle visited the premises October 14.

The variety of pansy being grown was "Oregon Giant." The seedlings, from home-grown seed sown on August 3, occupied one and a half frames (3 × 6 ft.). Seedlings on the more elevated ground at the edge of the frames became diseased first and had been removed several weeks before Jehle's visit. By this time, practically all plants in the frames were affected and many were severely stunted (Fig. 1, A–C; D, a and b, and E). The disease was clearly violet scab.

Her two pansy beds had been "disease ridden" in the spring of 1947 the grower told us. Before transplanting she had noticed that a few seedlings in the frames were unhealthy. She recalled, too, that about five years ago, when she was growing pansies under glass, this same trouble had been present. In earlier years, happily, this pansy grower appears to have been spared losses resulting from this cause.

    [3] Jenkins, A. E. "Oedema" or "wart" of cultivated violet identified as scab. Jour. Wash. Acad. Sci. 34: 352–357. 1944.
    [4] Jenkins, A. E., and A. A. Bitancourt. Spot anthracnoses in the United States and some island possessions. U. S. Dept. Agr., Plant Dis. Reptr. 31: 114–117. 1947.
    [5] Jenkins, A. E. Unusual collections of destructive fungi on plaintain and violet in the District of Columbia area. U. S. Dept. Agr., Plant Dis. Reptr. 24: 370–372. 1940.
    [6] Walker, E. A., R. A. Jehle, and A. E. Jenkins. Violet scab widely distributed in Maryland. U. S. Dept. Agr., Plant Dis. Reptr. 30: 471–474. 1946.
    [7] Massey, L. M., and A. E. Jenkins. Scab of violet caused by Sphaceloma. Cornell Univ. Agr. Exp. Sta. Mem. 170, 9 p., 1935.
    [8] Miss Sophia Grodzicka, to whom acknowledgment is made for her painstaking cooperation in furnishing these and other facts pertaining to her violet and pansy culture since 1928.
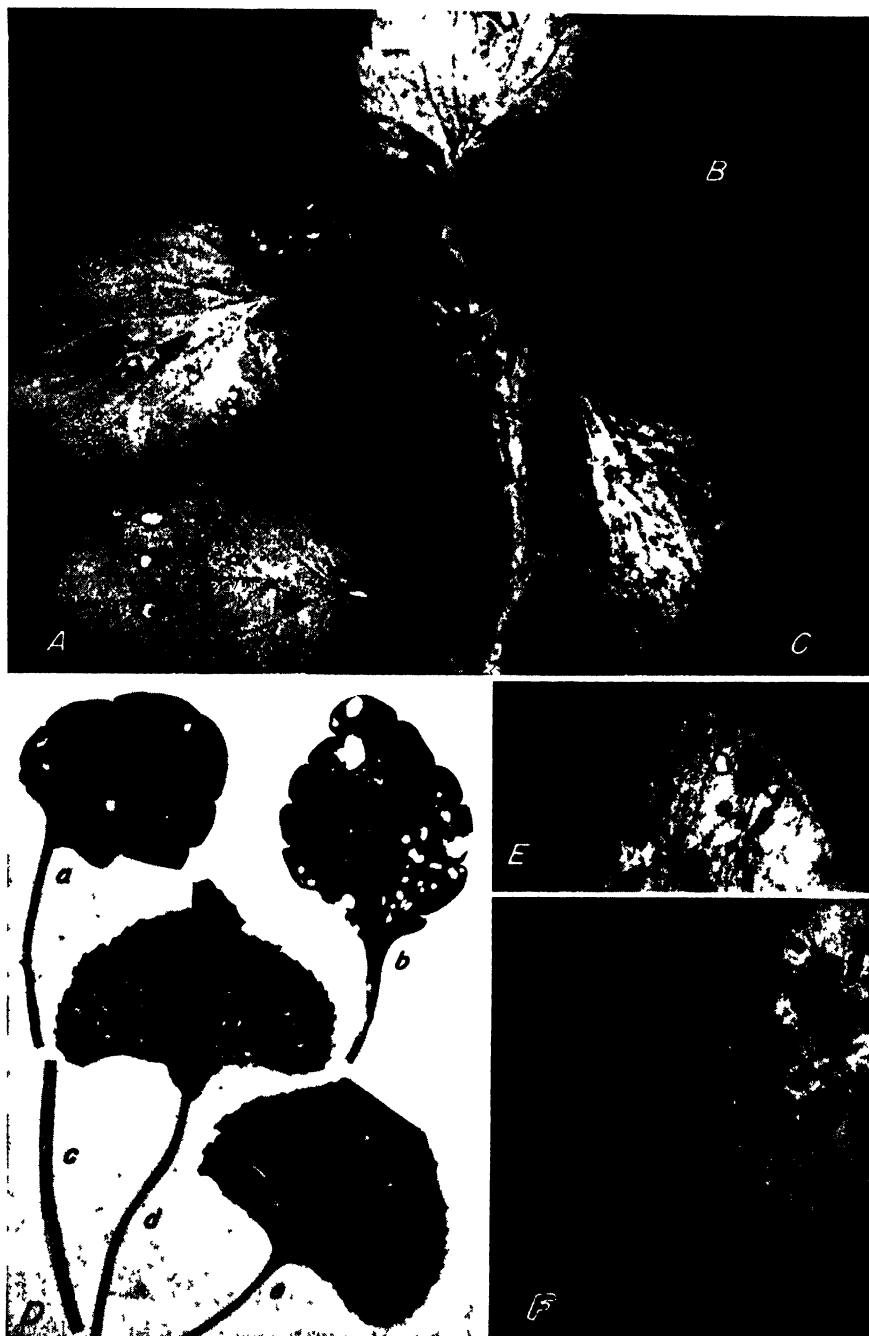
FIG. 1. A–C, D, *a* and *b*, and E. Growth of pansy seedlings affected by pansy scab, leaf surfaces in A, chiefly lower; D, *a*, upper and D, *b*, lower leaf surface. A–C and E, × 2; D, *a* and *b*, × 1; E, × 2.

· D, *c, d, e,* and F. Scab on petioles (D, *c, d, e*), and lower (D, *d* and F) and upper (D, *e*) leaf surfaces of wild violets growing on same property as the pansies. D, *c–e*, × 1; F, × 2; D, photograph of dry pressed specimens by R. L. Taylor, the others of fresh specimens by W. F. Jeffers.

The scab fungus could well have been transferred to her pansies from affected violets on the premises. Last autumn (1947) wild violet volunteers growing near the pansies, along paths leading to them, and elsewhere on the premises were abundantly diseased (Fig. 1, D, c-e, and F).

In their inoculation experiments more than a decade ago, Massey and Jenkins (loc. cit.) proved the susceptibility of pansy as well as cultivated and wild violets. No data were available on the natural occurrence of the disease on pansy, but a few instances have since been reported.[9] The recent severe cases of scab of pansy and violet growing in close proximity obviously afford implications of economic significance.—R. A. JEHLE, University of Maryland, College Park, Md. and ANNA E. JENKINS, U. S. Department of Agriculture, Beltsville, Md.

A Convenient Method for Isolating Slow-growing Pathogenic Fungi from Plant Tissues.—While studying the scab of camellias, a method was employed which proved to be very useful and convenient for isolating not only the fungus causing camellia scab but also for other slow-growing pathogenic fungi on other hosts (Table 1). The writer does not claim originality for this method but believes that it should be more generally used in pathological work. It represents a modification of the method described by Bitancourt[1] which was originally brought to the plant pathology laboratory of Louisiana State University by his colleague, S. C. Arruda,[2] while doing graduate work. As originally described by Bitancourt, the method consisted of cutting free hand (approx. $0.5 \times 0.3 \times 0.02$ mm.) sections of plant tissue through the lesion, or, more specifically, through the fruiting body of the pathogen with the aid of a dissecting microscope, and transferring these to agar drops on glass slides kept in humid atmosphere in Petri dishes. After a suitable period (24–48 hours) the sections on the agar drops were examined with the microscope, and those showing growth of what appeared to be *Sphaceloma* were transferred to plates or tubes.

The modification made by the writer consisted of placing the tissue sections on hanging drops of agar on cover slips suspended on glass rings in a moist chamber, instead of on slides.[3] This modification eliminates, to some extent, the danger of contamination as it provides a separate sealed chamber for each agar drop. A tissue section can be examined with the microscope through the glass cover slip as long as seems necessary without the risk of contamination from the air.

[9] Jenkins, A. E. Additional records of violet scab. U. S. Dept. Agr., Plant Dis. Reptr. 22: 86–88. 1938. Cf. also Whetzel, footnote 2.

[1] Bitancourt, A. A., and Anna E. Jenkins. Ciclo evolutivo de *Elsinoe australis* Bitancourt & Jenkins, agente da verrugose da laranja doce. Arq. Inst. Biol. S. Paulo 10: 129–146. 1939.

[2] Arruda, S. C. Sphaceloma diseases of camellia and other plants. Thesis, deposited in library, Louisiana State University. 1942.

[3] Hanna, W. F. A simple apparatus for isolating single spores. Phytopath. 18: 1017–1021. 1928.

Seven or eight holes are cut in two layers of filter paper of a size to fit the bottom of a 15-mm. Petri dish. Glass rings are placed over the holes and cover slips on the glass rings. The dishes thus prepared are sterilized dry in an autoclave. After the dishes have cooled, drops of melted agar are placed on the cover slips and enough sterile water poured into the dish to wet the filter paper. It is essential to wet the filter paper almost immediately after the agar drops have been deposited on the cover slips in order to prevent drying of the agar during the subsequent operations. Carrot agar[4] has been commonly used, but other media can be used. Bitancourt and Arruda used potato-dextrose agar, and the writer has also used potato-dextrose and oatmeal agars with success.

TABLE 1.—*Examples of successful isolation of slow-growing fungus pathogens by placing thin sections of diseased plant parts on hanging agar drops*

Kind of material	No. of sections plated	Pure cultures of desired pathogen	Various other fungi	Bacteria	No growth
A. Camellia scab (*Sphaceloma* sp.)					
1. Black spot type	20	6	7	0	7
2. Angular spot type	37	16	9	1	11
3. Zonate spot type	21	12	6	0	3
4. Corky excrescence	215	52	75	3	85
B. Chinese holly scab (*Sphaceloma* sp.)					
1. Black spot on leaves	83	8	19	0	56
2. Old leaf distorting lesion	20	6	9	0	5
3. Young do	35	12	9	0	14
4. Lesions on shoots	49	23	22	1	3
5. Lesions on berries	40	9	18	0	13
C. Dewberry anthracnose (*Elsinoe veneta*), lesions on canes	40	6	3	1	30
D. Strawberry scorch (*Diplocarpon earliana*)	51	30	7	4	10
E. Strawberry leaf-spot (*Mycosphaerella fragariae*)	49	8	0	1	40
Total	660	188	184	11	277

Materials (leaves, stems, berries) were washed thoroughly with a brush in running water, dipped in calcium hypochlorite solution for 1 to 2 minutes, and, without rinsing, dried between layers of sterile filter paper. Then a small piece containing a lesion was placed in pith and clamped tightly in a screw clamp. With relatively rigid material, like leaves of camellia or holly, the piece to be sectioned was often allowed to project 1 to 2 mm. above the pith thus lessening the chances of contamination from contact with the pith. Then, by holding the clamp with the left hand under the dissecting microscope, small slices of tissue (about 0.5 mm. × 25 μ) were sectioned with a sharp razor blade and transferred by means of the transfer needle to the agar drops. The cover slips were then lifted

[4] Approximately 300 gm. sliced carrots per liter of water, autoclaved for 20 minutes, strained through cotton, +20 gm. agar + 20 gm. sucrose.

with the forceps and reversed so that the agar drops hung within the glass ring. Precautions usually were taken to lessen the chances of contamination by flaming the blade lightly before cutting the section and also the needle before transferring the section to the agar drop. Actually, these precautions did not appear to be necessary. In several instances, the material to be sectioned was washed but not surface-disinfected, and in others not even washed, yet practically no bacterial contaminations occurred. Apparently the small size of tissue used greatly minimized the probability of a bacterial cell being lodged on it.

It was possible to examine the tissue pieces on the hanging drops with the microscope without exposing them to contamination and the development of the colonies was followed. By adding sterile water to the dish to prevent drying, the cultures were kept as long as desired. Usually, 48 hours were sufficient for *Sphaceloma* colonies to be recognizable and ready for transfer.—A. G. PLAKIDAS, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Local Lesions with Potato Virus Y.—In an attempt to find a satisfactory local lesion host for potato virus Y (*Marmor upsilon* Holmes),[1] over a hundred plant species were tested for their reaction to mechanical inoculation with this virus. In general the plants were used after the older leaves had attained maximum size but before flowering had begun. The leaves were dusted lightly with 400-mesh carborundum and then rubbed with a gauze pad saturated with freshly expressed juice from a diseased *Nicotiana glutinosa* L. plant. Four new local lesion hosts were found, *Chenopodium urbicum* L., *Physalis floridana* Rydb.,[2] *Lycium halimifolium* Mill., and *L. chinense* Mill. There is some doubt, however, as to whether or not the two species of *Lycium* are distinct from the *L. barbarum* reported by Dennis[3] as a local lesion test plant for virus Y.

Pale green to whitish-yellow non-necrotic lesions appear on the inoculated leaves of *Chenopodium urbicum* 14–16 days following inoculation (Fig. 1, A). Distinct brown necrotic lesions develop on inoculated leaves of the Lycium species in about 10 days (Fig. 1, B). Infection is restricted to the inoculated leaves in both Lycium species and in *C. urbicum*. Small necrotic spots appear on inoculated leaves of *Physalis floridana* about 9–10 days following inoculation (Fig. 1, C). The lesions enlarge and finally coalesce. At this stage the leaves show epinasty and begin to abscise acropetally. Concurrently, systemic symptoms appear, consisting of rugosity and necrotic spotting of the young leaves followed by leaf abscission. Lesions on inoculated leaves can be counted by about the 12th day.

Vigna sinensis Endl.[4] and certain potato (*Solanum tuberosum* L.) varie-

[1] Potato veinbanding strain. Kindly supplied by E. S. Schultz.
[2] Identified by Dr. W. C. Muenscher.
[3] Dennis, R. W. G. A new test plant for potato virus Y. Nature **142**: 154. 1938.
[4] Chester, K. S. Serological evidence in plant virus classification. Phytopath. **25**: 686–701. 1935.
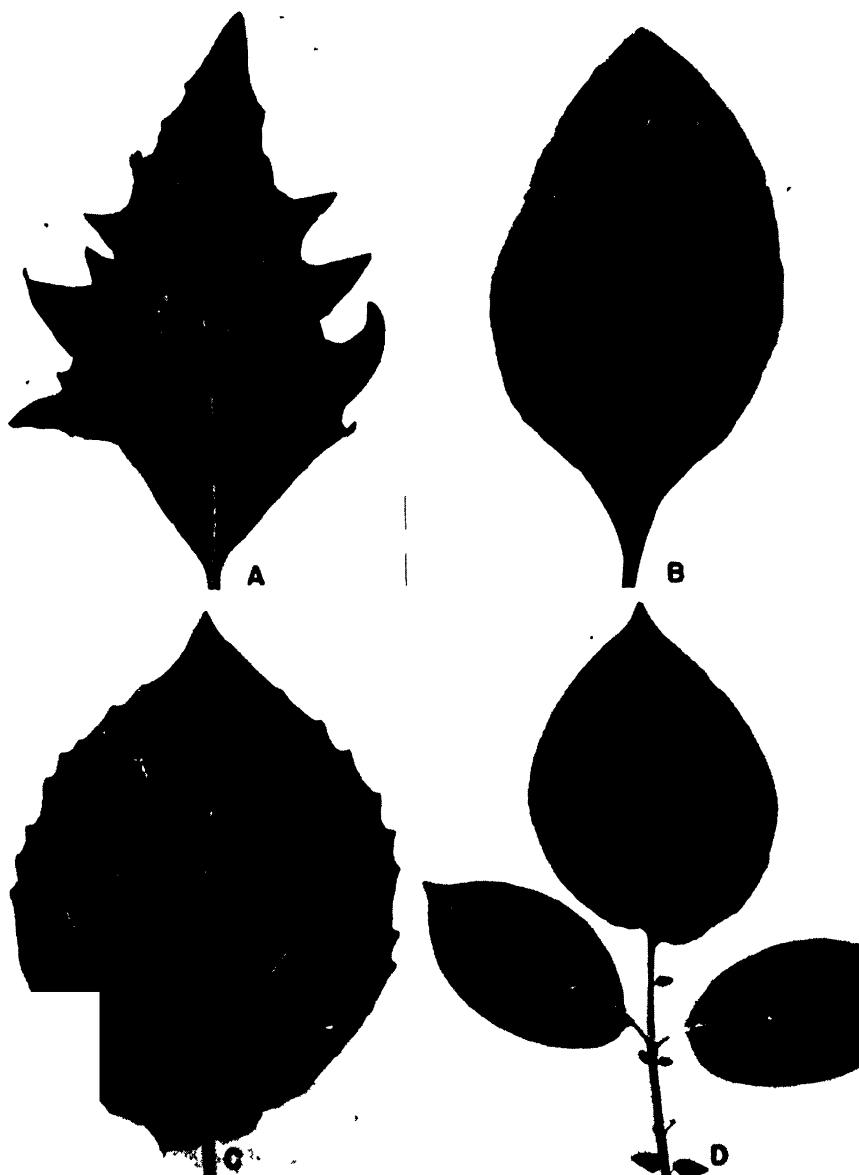
FIG. 1. Local lesions on leaves inoculated with potato virus Y. A. *Chenopodium urbicum* (×1). B. *Lycium halimifolium* (×¾). C. *Physalis floridana* (×½). D. U.S.D.A. potato seedling 41956 (×½).

ties[5] and seedlings[6] have been reported as reacting to mechanical inoculation with potato virus Y by the formation of local lesions. In further tests, *V.*

[5] Dykstra, T. P. A study of viruses infecting European and American varieties of the potato Solanum tuberosum. Phytopath. 29: 40–67. 1939.

[6] Hutton, E. M., and J. G. Bald. The relationship between necrosis and resistance to virus Y in the potato. 1. Greenhouse results. Jour. Coun. Sci. Indus. Res. (Aust.) 18: 48–52. 1945.

sinensis, U.S.D.A. potato seedling 41956,[7] *Chenopodium urbicum, Physalis floridana,* and *Lycium halimifolium* were compared as test plants for quantitative work with virus Y. Carborundum was used in all inoculations. No lesions were obtained on *V. sinensis.* The dilution curves obtained on each of the other test plants indicated that all four can be used for quantitative work. *L. halimifolium* was not included in subsequent tests because of its undesirable growth habits. Virus solutions differing in concentration by 20 per cent generally gave a significant difference in number of lesions on *P. floridana, C. urbicum,* and the potato seedling when 48 or more leaves or half-leaves were inoculated with each solution. Modified half-leaf techniques were used with *P. floridana* and seedling 41956 and a randomized arrangement of the inoculated leaves with the other species. In these tests juice of diseased *N. glutinosa* plants was diluted 1–100 or 1–200 when *P. floridana* was inoculated and 1–25 or 1–50 when either of the other two was inoculated.

Under most conditions *Physalis floridana* has proved to be the best of the local lesion plants tested. The leaves are well suited for the half-leaf technique. Plants react satisfactorily over a rather long period, beginning just prior to flowering and continuing until the leaves begin to yellow. Hence it is possible to use plants with as many as 16 large leaves. A given inoculum produces from 4 to 10 times as many lesions on *P. floridana* as on any of the other species. Juice of diseased *Nicotiana glutinosa* plants diluted 1–100 will produce from 20 to 200 lesions per half-leaf. Scattered lesions are not infrequent at dilutions of 1–10,000.

Physalis floridana cannot be used under all conditions. Necrotic lesions do not develop satisfactorily when the plants are held near 80° F. or above. Some strains of potato virus X (*Annulus dubius* Holmes) produce lesions on this species (also on *Chenopodium urbicum* and the Lycium species) similar to those caused by virus Y. Preliminary tests with potato seedling 41956, which is immune from virus X, indicate that it may be useful not only for quantitative work with naturally infected potatoes (where virus X may be present) but also for indexing work. Juice of triturated shoots or sprouts from Y-infected potatoes, diluted 1–5 or 1–10, regularly caused the formation of many lesions when rubbed on the leaves of this seedling.

Other experiments showed that *Physalis floridana* can be used where virus X is present. Plants inoculated when young with a mild strain of virus X react to subsequent inoculation with virus Y by the formation of as many or more lesions than will corresponding healthy plants inoculated with virus Y. Mild strains of virus X produce no marked symptoms and protect the plant against all tested strains of virus X.—A. Frank Ross, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Longevity of Fungus Cultures under Mineral Oil.—In 1946 the senior author[1] reported on the two-year survival of *Diplodia zeae, Gibberella zeae,*

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[7] Found to produce necrotic lesions following mechanical inoculation with virus Y (Fig. 1, D) by Dr. W. F. Mai. Personal communication, 1946.

[1] Wernham, C. C. Mineral oil as a fungus culture preservative. Mycologia **38**: 691–692. 1946.

Helminthosporium maydis, and *Nigrospora oryzae* placed under mineral oil March 15, 1944.

Since the appearance of this brief note Buell and Weston[2] have reviewed the pertinent literature and have presented considerable data pertaining to the preservation of a number of fungi on media submerged under mineral oil. In addition Edwards, Buell, and Weston[3] have published data from which they deduce that the prolonged life, without transfer, of *Sordaria fimicola* under mineral oil is due to a reduction in oxygen consumption.

Although Henry[4] has reported good growth with unchanged morphology of seventeen wine yeasts held seven years under mineral oil, data on other fungi are not available for periods longer than three and one-half years. Little, too, is known about preservation of pathogenicity.

On March 15, 1948, four years after treating them with mineral oil, the four maize pathogens[1] were transferred to standard Difco potato-dextrose agar. The *Diplodia* culture had been used as a stock culture so frequently that only portions of the agar substrate could be transferred. Mycelium appeared to be wholly depleted: the other three cultures still yielded plenty of hyphal material for transfer.

Within three days luxuriant growth of all but *Diplodia* had taken place. Within nine days two of four *Diplodia* transfers gave good cultures but the remaining two did not grow. These subcultures definitely establish a length of survival of four years.

During the 1947 season *Diplodia, Gibberella,* and *Helminthosporium maydis* were tested for pathogenicity. All appeared to be actively pathogenic after three years under oil.

Following the appearance of the original note in Mycologia, Dr. W. R. Goss, in correspondence, pointed out that he had used the mineral oil method but had found it unsatisfactory for pathogenic Phycomycetes held at room temperature.

The junior author investigated the behavior of *Phytophthora infestans* which is used in this laboratory as a standard for testing fungicides. Forty-five cultures on Lima-bean agar were treated and held at 12° C. At the end of one year, only two were nonviable. Twenty-eight of the forty-three remaining ones were subcultured, and the twenty-eight original as well as the subculture tubes were held under mineral oil for an additional year. Twenty of the two-year tube cultures survived; all of the one-year subcultures survived. Pathogenicity of the two-year survivors was reduced but not lost when tested on potatoes grown in the greenhouse.

[2] Buell, C. B. and Wm. H. Weston. Application of the mineral oil conservation method to maintaining collections of fungus cultures. Amer. Jour. Bot. 34: 555–561. 1947.

[3] Edwards, G. A., Caroline B. Buell, and Wm. H. Weston. The influence of mineral oil upon the oxygen consumption of *Sordaria fimicola*. Amer. Jour. Bot. 34: 5J1–555. 1947.

[4] Henry, B. S. The viability of yeast cultures preserved under mineral oil. Jour. Bact. 54: 264. 1947.

Sclerotinia fructicola and *Venturia inaequalis* have also been kept satisfactorily for two years without transfer but no test was made of pathogenicity.—C. C. WERNHAM and H. J. MILLER, Department of Botany, The Pennsylvania State College.

Curly Top of Muskmelon.—Severin[1] reported finding muskmelon naturally infected with curly-top virus during 1925 and 1928. The disease has been destructive to the commercial crop in the west central and southern portions of the San Joaquin Valley of California during some seasons. It was also reported serious on the early plantings in the Salt River Valley area of Arizona during the 1947 season.

Diseased plants in the cotyledon and the one or two-leaf stage (Fig. 1, A)
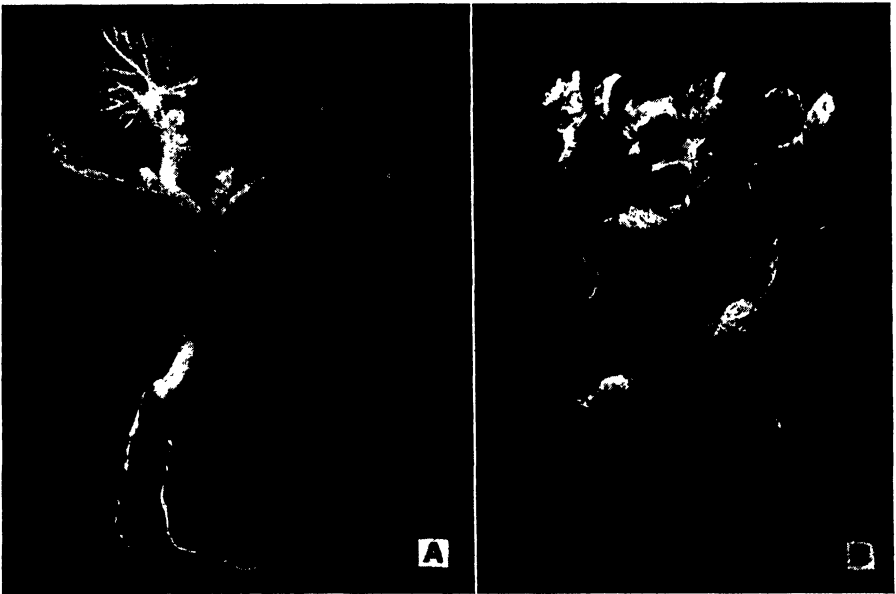
FIG. 1 A. Curly-top diseased muskmelon plant from Arizona, with distorted leaves and prominent veins. B. Portion of a Persian melon plant from a commercial field near Murray, California, showing dwarfing and distortion as a result of curly-top infection.

were sent by Orin A. Hills, Bureau of Entomology and Plant Quarantine, from Arizona about mid-April, 1947, and curly-top virus was recovered from them. Another shipment of plants from the same field was received about mid-June. These plants, with runners a foot or two long, were appreciably stunted and distorted, and curly-top virus was recovered from them. The melon commonly grown in this area of Arizona is known as the Mildew Resistant 45 and the plants tested were reported to be that variety. Diseased cantaloupe plants grown near Murray, California, were collected May 21, 1947, by Dr. Eubanks Carsner, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering. These

[1] Severin, H. H. P. Some host plants of curly top. Hilgardia 3: 339–384. 1928.

plants were infected by the curly-top virus and had runners one to two feet long. Plants of Persian melon from the same area were collected by Dr. Carsner August 13, 1947, and at the time were carrying mature melons although they were much dwarfed and distorted. Practically all plants in the field were reported as showing good curly-top symptoms (Figure 1 B).

A few greenhouse inoculations of young melon plants made using one or two leafhoppers per plant resulted in a comparatively small percentage of infection, while greater numbers of leafhoppers gave a high percentage of infection. These results, and the fact that several plants escaped infection with the heavier inoculations, indicate that muskmelon seedlings are dis-

FIG. 2. Mildew Resistant 45 muskmelon variety. Two dwarfed plants are infected by curly-top virus strain 10 and large plant is not infected. Cotyledons on infected plants are light yellow. Photographed 23 days after inoculation.

tinctly more resistant to curly-top infection than are young sugar beet plants.

In order to learn more concerning the susceptibility of muskmelon varieties to curly top, greenhouse inoculations were made on plants of the following varieties: Rocky Ford, Tip Top, Honey Rock, Hearts of Gold, Japanese melon, Honey Dew, Cranshaw, Golden Beauty Improved Casaba, Golden Hybrid Casaba, Persian, Hale's Best 45, and variety 45 from four other sources. All were started in 6-inch pots but a few were transferred to 10-inch pots to see the influence of greater opportunity for root growth. Most of the plants in each group were inoculated in the young cotyledon stage, using 15 to 20 leafhoppers to a group of three plants and a large

cage which covered all the plants. Some of each group were inoculated, using the small leaf cages with two or three leafhoppers to a cage and three or four cages on each plant. Infections were obtained equally well with both types of cages. Inoculations were made with virus strains 1, 2, 3, 4, 5, 6, 8, 9 and 10, as well as some selections of curly-top virus from the field. Infection was obtained with each of the virus strains except 2 and 4. Only plants of the Gem variety were inoculated with strain 4. Numerous attempts to recover curly-top virus from plants inoculated with strains 2 and 4 gave negative results. This would indicate that the less virulent strains 2 and 4 do not infect these melons.

Although infection was rather general among the melon varieties, the apparent amount of injury differed greatly. Ten days to two weeks after inoculation there was distinct yellowing of the cotyledons on many of the plants (Fig. 2) in the four lots designated as "mildew resistant 45", Hale's Best 45, Honey Rock, Rocky Ford, and Hearts of Gold. Three weeks after inoculation the four lots of Mildew Resistant 45, Hale's Best 45, and Honey Rock were easily grouped as the ones most severely injured, while the casabas and Cranshaw appeared to be least injured. A high percentage of the plants showing definite symptoms were severely dwarfed (Figure 2) and many of these died. Some infected plants lived for more than five months after inoculation and curly-top virus was readily obtained, five months after inoculation, from one dwarfed plant infected with strain 1. Most such plants died within two or three months. One plant infected with strain 10 virus grew quite normally and had a runner over ten feet long, but virus was readily obtained from ·it four months after inoculation. Nearly 200 tests were made from the different melon varieties to determine the presence of curly-top virus in them.

It is clearly evident that the curly-top virus may induce serious injury in commercial plantings of muskmelons, and serious losses were reported for certain areas in the western San Joaquin Valley of California and the Salt River Valley of Arizona during 1947. Some of the evidence secured in greenhouse experiments suggests the possibility that there may be opportunity for selection and breeding of muskmelon for resistance to curly-top.—N. J. GIDDINGS, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

A METHOD FOR MEASURING RESISTANCE TO DEFOLIATION DISEASES IN TOMATO AND OTHER LYCOPERSICON SPECIES[1]

SETH BARTON LOCKE[2,3]

INTRODUCTION

Two defoliation diseases of tomato, namely, early blight caused by *Alternaria solani* (A. and M.) Jones and Grout, and septoria leaf spot, caused by *Septoria lycopersici* Speg., are commonly destructive east of the Rocky mountains. Early in 1939, a project was undertaken at the Arkansas Agricultural Experiment Station which had for its goal the development of horticulturally superior strains of tomato possessing resistance to these two diseases. As the first step toward this goal it was necessary to devise a technique for accurately measuring resistance to the fungi in question. Various procedures have been used for this and similar purposes in the past.

The literature contains numerous reports (1, 2, 5, 7, 9, 12, 13, 17, 19, 20) which compare tomato varieties exposed under field conditions to natural infection by the organisms named. These tests have not been entirely satisfactory because the principal environmental factors influencing infection and symptom expression were uncontrolled, and because several pathogens often attacked the plants simultaneously. Artificial inoculation with pure cultures of the pathogens under partially controlled conditions in the greenhouse or inoculation chambers (1, 3, 8, 10, 15) has overcome some of the difficulties which are inherent in the field test. The procedure described here is of the latter type, and its distinctive features are (a) the use of detached leaf material for inoculation, (b) inoculation with pure cultures of the pathogens, and (c) evaluation of host reaction on the basis of lesion diameter. A preliminary report (11) on this procedure was made in December, 1941.

Extensive use of detached leaves has been made by others in studies involving obligate parasites and to a lesser extent with facultative parasites. A review of the literature on detached leaf culture including application to studies on susceptibility of hosts and virulence of pathogens has been published recently (21). Detached tomato leaflets were used by Douglas (6) to compare the response of tomato varieties to inoculation with an undetermined species of *Alternaria*. Bonde used a similar method to compare strains of *A. solani* with respect to virulence on potato. More recently

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Wellman (18) reported that the use of excised tomato leaflets for comparing virulence of strains of *A. solani* on tomato was unsuccessful because of rapid breakdown of the tissues caused by secondary organisms.

PROCEDURE

Plant Material

Plant material was selected for uniformity in age and absence of disease and insect injuries. The best results were obtained when young, vigorous plants, 8 to 10 inches tall, were used, but satisfactory results were also obtained with material from older plants, provided the plants were growing vigorously. The leaflets were taken from near the top of the plants, preference being given to leaves that were fully expanded but which still showed no signs of senescence. Usually the three apical leaflets of a leaf were taken but occasionally, where the leaflets were small, it was necessary to take five leaflets in order to provide space for the necessary number of inoculations. These were placed immediately in individual moist chambers consisting of 10-cm. Petri dishes whose covers were lined with two layers of moistened filter paper. Care was taken to avoid wilting because wilted leaflets did not regain turgidity after being placed in the moist chambers.

Cultures

The pathogens were originally isolated from naturally infected tomato leaves by transferring single spores to potato-dextrose-agar plates, where the early stages of growth were observed under low magnification in order to make certain that no contaminants were present. Cultures of *Septoria lycopersici* appeared to be fairly stable with regard to cultural characteristics and virulence. *Alternaria solani*, when freshly isolated, on potato-dextrose agar, produced the wine-red pigment described in the literature as characteristic of the species (4, 14, 16), but later gave rise to non-pigmented sectors. Only pigmented cultures were used for inoculum. Fresh isolations were made from naturally infected tomato foliage each season, and transfers were made only from pigmented sectors of the parent cultures. It has been shown that differences in virulence exist among different isolants of *A. solani* (18). However, this could not be a source of error in these tests because identical inocula were used for all comparisons between strains of the host plant.

Alternaria solani was cultured on potato-dextrose-agar plates, a single planting being made at the center of the plate. Greatest uniformity of growth was obtained when the medium was rather deep (5 mm.). The cultures were kept at room temperature in diffused light until the growth had nearly covered the plate, when the cultures were ready for use in the preparation of inoculum. *Septoria lycopersici* pycnospores were planted thickly over the entire surface of potato-dextrose-agar slants. Numerous, competing colonies developed and gave rise to an abundance of pycnidia and pyc-

nospores after from seven to eight days at room temperature in diffused light.

Preparation of Inoculum

Inoculum consisted of a suspension of pycnospores in the case of *Septoria lycopersici* and of mycelial fragments in the case of *Alternaria solani*.[4] The *A. solani* inoculum was prepared by macerating an entire plate culture in 50 cc. of sterile soil extract[5] in a mechanical blender (3). The *S. lycopersici* inoculum was prepared by flooding the surface of a slant culture with 10 cc. of sterile soil extract and agitating for a few minutes by rolling the tube between the hands. The resultant spore suspension was poured off and made up to 50 cc. with additional soil extract.

Inoculation and Incubation

Inoculation with mycelial or spore suspensions was accomplished by placing a droplet of the suspension on the lower surface of the tomato leaflet using a 3-mm. loop. A needle puncture was then made in the leaf tissue directly beneath the drop of inoculum. Inoculation without wounding often resulted in delayed infection, infection at several points around the periphery of the inoculum droplet, or in no infection. Wounding insured immediate and localized infection. The test is thus a measure of resistance to invasion as distinct from resistance to entry.

Ten inoculations were made on the leaf material in each moist chamber. Each plant was represented by the leaf material in one moist chamber in each of five series of inoculations. Thus, there was a total of fifty inoculations for each plant included in the test. Inoculation of any one series was completed on the same day that it was started, but different series were frequently inoculated on different days.

Inoculated leaflets were incubated at room temperature in diffused sunlight, for three days in the case of *Alternaria solani,* and for eight days in the case of *Septoria lycopersici*. Attempts to incubate the leaves in darkened chambers under controlled temperature resulted in the leaves becoming etiolated and subject to rotting by other organisms.

Evaluation of Host Response

Evaluation of resistance or susceptibility was based upon the relative sizes of the lesions resulting from inoculation. Each lesion was compared with a graded series of circular spots having known diameters and arranged for convenience along the edge of a card. The diameter of the circular spot corresponding most closely in area to the lesion was recorded.

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[4] In some of the early tests small, 2×2 mm., blocks were cut from the plate culture of *A. solani*, and these were used to inoculate the leaflets by placing them fungus-side-down on the lower surface of the leaflets. This procedure was later abandoned in favor of the less tedious use of the mycelial fragment suspension.

[5] The soil extract was prepared by shaking 500 gm. greenhouse compost with 1000 cc. distilled water, allowing the soil to settle, decanting the clear extract, and making up to 1000 cc. with distilled water. Spores of *A. solani* and *S. lycopersici* germinated better in soil extract than in tap or distilled water.

In making comparisons the lesion was viewed from the upper surface of the leaflet, that is, from the side opposite the one on which the leaf was inoculated. In many cases the lesions were not circular, but it was nevertheless a simple matter to select a standard spot having nearly the same area. Occasionally the leaflets became watersoaked beyond the area occupied by the lesion. By holding the leaflet before a strong light, the approximate limits of the lesion could be determined.

The basic data consisted of the averages of the diameters of the ten lesions in each of the moist chambers. Thus, there were five such mean lesion diameters for each plant in the test, one in each of five series. These were subjected to an analysis of variance in which the variabilities associated with series and with strains of plant material were segregated. The residual variability was used to estimate experimental error. This experimental design is similar to the completely randomized block design commonly used in field experiments, the different inoculation series corresponding to the blocks in the field experiment.

TABLE 1.—*Efficiency of the detached-leaf method for measuring resistance to Alternaria solani and Septoria lycopersici in Lycopersicon species*

Pathogen and test	Material tested	Strains	Mean lesion diameter		
	Species		LDRS[b]	D[c]	D/LDRS[d]
Alternaria solani		*number*	*mm.*	*mm.*	
2	*L.*[a] *esculentum*	9	2.00	2.74	1.37
4	do	11	1.68	1.88	1.12
2	*L. glandulosum*	5	2.02	2.06	1.02
2	*L. hirsutum*	7	2.37	4.34	1.83
4	do	8	2.59	3.60	1.39
2	*L. peruvianum*	8	1.28	2.40	1.88
4	do	12	2.15	2.16	1.00
2	*L. pimpinellifolium*	22	1.66	2.34	1.41
4	do	20	1.81	1.86	1.03
5	Tomato varieties	15	2.38	3.64	1.53
6	do	15	4.43	5.46	1.23
7	do	15	0.91	1.32	1.45
8	do	17	1.91	1.94	1.02
Septoria lycopersici					
1	*L. esculentum*	8	0.96	1.42	1.48
3	do	9	1.32	1.18	0.89
1	*L. glandulosum*	5	0.74	1.02	1.38
1	*L. hirsutum*	7	0.36	1.72	4.78
3	do	7	0.29	0.40	1.38
1	*L. peruvianum*	11	0.98	1.24	1.27
3	do	11·	1.96	1.58	0.81
1	*L. pimpinellifolium*	16	1.16	1.92	1.66
3	do	18	1.31	2.18	1.66
1	Tomato varieties	6	1.13	0.82	0.73
3	do	6	1.47	1.78	1.21

[a] *Lycopersicon.*
[b] Least difference required for significance at odds of 19:1.
[c] Greatest difference observed.
[d] Ratio of greatest observed difference to least difference required for significance at odds of 19:1.

DISCUSSION

The procedure described has a number of advantages over field tests and many of the greenhouse and laboratory tests previously employed. Some of these are as follows:

1. It provides for control of the important environmental factors which may influence the virulence of the pathogen and the reaction of the host.

2. It enables the testing of host reaction to a single pathogen' or a single strain of the pathogen without the confusion resulting from the activities of additional pathogens as is often the case with field tests. Furthermore, it is possible to test individual host plants independently for reaction to two or more different pathogens. Very susceptible plants are not eliminated and are available for genetic studies or testing against other pathogens.

3. The test is independent of such factors as growth habit and fruit load which affect the reactions of plants in field tests.

4. The test measures physiologic resistance to invasion irrespective of resistance to entry, and can be altered easily to give a measure of the latter.

5. The use of lesion diameter as a measure of host reaction reduces the personal factor to a minimum and permits statistical treatment of the data.

In the course of four years, this test has been applied to a wide range of plant material, including commercial tomato strains, other *Lycopersicon* species, and a *Lycopersicon* species-hybrid as well as some of its backcrossed and selfed progenies. Its use has facilitated the discovery of a source of practical resistance to both of the diseases in question, and it has aided progress toward the goal of incorporating genetic factors controlling such resistance into horticulturally superior tomato strains. Reference to table 1 will give the reader an indication of the magnitude of the differences in mean lesion diameter obtained in representative tests and how they compare with the least differences required for significance.

SUMMARY

A laboratory test was devised for measuring the relative resistance or susceptibility of tomato strains and *Lycopersicon* species to two commonly destructive defoliation diseases, namely, early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*).

The test makes use of artificial inoculation with pure cultures of the pathogens into detached leaflets which are then incubated in moist chambers. Evaluation of resistance or susceptibility is based upon lesion diameter attained during a uniform period of incubation.

The test has proven sufficiently sensitive to show small but significant differences in susceptibility to these diseases among commercial tomato strains. It has been useful in the discovery of a source of practical resistance to early blight and septoria leaf spot and has aided in progress

toward incorporating genetic factors controlling this resistance into horticulturally superior tomato strains.

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CONTROL OF COTTON WILT AND NEMATODES WITH A SOIL FUMIGANT[1]

A. L. SMITH[2]

INTRODUCTION

Cotton wilt caused by *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen, which occurs widely in southeastern and south central United States, is usually controlled by planting resistant varieties. The root-knot nematode, *Heterodera marioni* (Cornu) Goodey, is commonly associated with cotton wilt in light soils. It is generally recognized that cotton wilt tends to increase in the presence of root-knot nematode. Taylor and associates (5, 6) and Smith (2) have mentioned the meadow nematode, *Pratylenchus pratensis* de Man, as an important factor in increasing wilt and reducing yields. Subsequent observations by the writer suggest that the meadow nematode is of considerable importance in fields where wilt becomes severe and where resistant varieties become wilted and stunted and do not produce satisfactorily. The present study is concerned with attempts to control this disease complex with a soil fumigant. Dowfume W-10[3] was used in all studies reported here.

LITERATURE REVIEW

The possibility of controlling the wilt-nematode complex was demonstrated by Taylor and associates (5, 6). In their studies made at Lumberton, North Carolina, several varieties were compared on soil treated with carbon-disulphide and on non-treated soil. They found that the carbon-disulphide treatment controlled nematodes sufficiently to enable various wilt-resistant and wilt-tolerant varieties of cotton to become established and grow satisfactorily. Only a moderate amount of wilt developed in treated plots. A parallel situation was reported by Young (7) with tomato wilt. The application of 300–600 lb. chloropicrin per acre delayed the appearance of wilt symptoms 20–40 days and reduced the wilted plants to 7–10 per cent in the treated plots, while there were from 72 to 100 per cent wilted plants in non-treated plots. Godfrey (1) and several later investigators have demonstrated the fungicidal properties of chloropicrin. The fungicidal properties of ethylene dibromide used in the present study

have not been thoroughly investigated, although Stark and associates (4) found ethylene dibromide ineffective in controlling damping-off of pea seedlings in greenhouse studies.

FUMIGATION OF MODERATELY INFESTED SOIL

In 1946, a test was run at Plains, Georgia. The soil was a Greenville sandy loam of low productivity with a moderate wilt, root-knot, and meadow nematode infestation. The fumigant was applied April 4 at the rate of 31 gallons per acre. The test consisted of 4 replications with rows 70 feet long. The rows were 42 inches wide in all tests reported here. The fumigant was injected to a depth of 6 inches at points 18 inches apart. One line of injection points was placed directly in the row to be planted and these were staggered with a second line of points between the planted rows. A non-treated buffer row was included between treated and non-treated plots. Coker 4 in 1–7, a wilt-resistant and root-knot tolerant variety, was compared with Deltapine 14, a variety susceptible to both wilt and root knot. Treated plots alternated with non-treated control plots.

The plantings were made April 9 under favorable conditions. A slight phytocidal action of the Dowfume W-10 was apparent by the reduced number of seedlings. The differences in emergence on treated and non-treated plots were not great. A satisfactory stand of Deltapine and a poor stand of Coker 4 in 1–7 were obtained. Wilt data were recorded by removing dead plants at intervals and by counting the remaining wilted plants and non-wilted plants at the end of the season. After picking the cotton, the roots were examined and root-knot indices determined according to the method described by Smith and Taylor (3).. The results are shown in table 1.

TABLE 1.—*The effect of soil treatment with Dowfume W-10 on the yield, percentage of wilt, and the root-knot index of cotton varieties grown at Plains, Georgia, in 1946 and at Tallassee, Alabama, in 1947*

Location and variety	Treatment rate (Gal. per acre)	Lint yield per acre (Lb.)		Percentage increase in yield from treatment	Wilted and dead plants (Per cent)		Root-knot index	
		Con.[a]	Treated		Con.	Treated	Con.	Treated
Plains, Ga.								
Coker 4 in 1–7	31	197	281	42	2.6	0.5	50.2	2.7
Deltapine 14	31	162	336	108	21.3	0.9	96.8	6.9
Tallassee, Ala.								
Coker 4 in 1–7	37.5	367	1067	191	52.7	1.8	95.7	12.6
	12.5	495	963	95	37.7	6.4	91.1	57.8
Deltapine 14	37.5	64	904	1312	96.8	3.2	100.0	33.9
Maretts C 4–8	12.5	593	942	59	16.4	2.5	90.0	68.9
Cook 142–10	12.5	465	762	64	6.2	3.3	96.7	70.8
Dixie Triumph 366	12.5	392	832	112	20.0	3.3	100.0	89.7
Miller 610	12.5	295	862	192	63.9	6.3	100.0	96.9
Hurley's Rowden	12.5	157	594	279	84.3	38.0	100.0	98.9

[a] Controls (Con.) received no treatment.

There was an increased yield from treatment and almost complete control of wilt and root-knot. Growth differences began to appear early in the season, particularly with Deltapine. After 8 weeks, plants in treated rows were 3 inches taller than those on non-treated plots; and differences became greater as the season progressed. The yield differences in this and later tests were accounted for largely by number of bolls, although the bolls and seed were larger and the lint indices higher from treated plots.

FUMIGATION OF HEAVILY INFESTED SOIL

The study was repeated, with minor variations, at Tallassee, Alabama, in 1947. The soil used at Tallassee is a Cahaba fine sandy loam, deep phase. Cotton has been grown on this particular area for several years
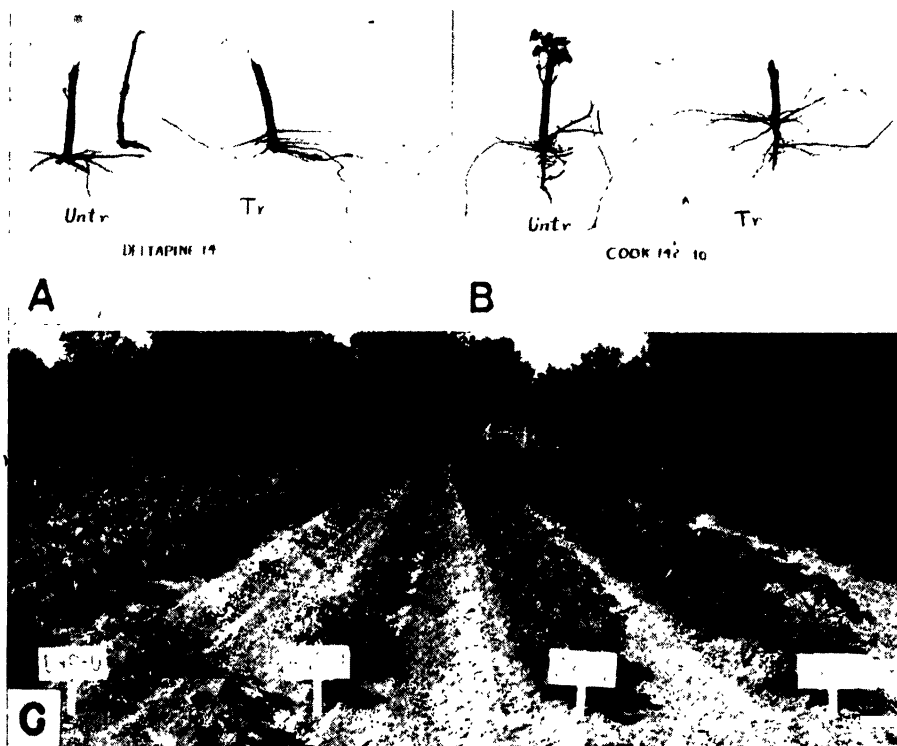
FIG. 1. A. Roots of Deltapine 14 cotton from untreated and treated plots; rate 37.5 gallons per acre. B. Roots of Cook 142–10 from untreated and treated plots; rate 12.5 gallons per acre. C. Early killing and stunting in untreated plots compared with vigorous growth in treated plots. Tallassee Test I, photographed July 10.

and the infestation of wilt, root-knot, and meadow nematode was heavy. Only the more resistant varieties can survive, and even these become stunted and wilted, and production is reduced. Otherwise, the soil is considered one of the best in Alabama for cotton production. The same varieties, Deltapine 14 and Coker 4 in 1–7, were planted. The test con-

sisted of 6 replications, with rows 70 feet long. The two treated rows were alternated with two non-treated rows to make up a replication. The fumigant was injected April 26 at a depth of 6 to 7 inches at staggered points 15 inches apart at the rate of 37.5 gal. per acre. The center row of injection points was in the row to be planted, with a row of injections 12 inches distant on each side. A guard row was not used between treated and non-treated rows and there was no evidence that the effect of the fumigant extended beyond the rows treated. The seed were planted under favorable conditions May 6, and an excellent stand was obtained for both varieties. Soil temperatures were not available for the period between soil treatment and planting, but the mean maximum and minimum air temperatures were 79° and 62° F., respectively. From this it may be assumed that the soil temperatures were favorable for the action of the fumigant.

Differences in rate of plant growth and amount of wilting were apparent early in the season (Fig. 1, C). Yields of lint were increased from 367 to 1067 lb. for Coker 4 in 1-7 and from 64 to 904 lb. for Deltapine 14. These yields represent increases of 191 and 1312 per cent, respectively. The wilt percentage was reduced from 52.7 to 1.8 and from 96.8 to 3.2 for the respective varieties. Wilt severity in this test is indicated by the high incidence in Coker 4 in 1-7, which is considered a relatively resistant variety in most soils. The root-knot indices were recorded November 26 and represent the seasonal build-up of the infestation. Plants in treated plots had normal appearing roots, indicating good control of both species of nematodes (Fig. 1, A). Such galls as were present were small and probably resulted from relatively late invasion of roots.

REDUCTION IN AMOUNT OF FUMIGANT

To determine the effect of a lower rate of application of the fumigant, an additional test was conducted at Tallassee, Alabama, in 1947. The rate used in this test was 12.5 gal. per acre. The material was injected to a depth of 6 to 7 inches at intervals of 15 inches in the row only. The varieties were randomized in 6 replications. A plot consisted of one row, 40 feet long. The number of varieties was increased to 6. Three varieties, Marett's C 4-8, Cook 142-10, and Coker 4 in 1-7 are considered wilt-resistant, and they have the best root-knot tolerance available in upland cotton varieties. Dixie Triumph 366 is resistant to wilt but rather susceptible to root-knot. Miller 610 is moderately wilt-tolerant and susceptible to root-knot. Hurley's Rowden is highly susceptible to both wilt and root-knot.

The yield data (Table 1) show much the same response to treatment as was obtained with the heavier application used at Tallassee. For the variety Coker 4 in 1-7, which was planted in both tests, the mean difference between plots in the two tests, 104 lb., may be partially attributed to

response to the heavier treatment. Plants were larger and grew more vigorously on the plots treated at the rate of 37.5 gallons.

The yields of other varieties having some wilt resistance were comparable to those of Coker 4 in 1–7 in treated plots. The small differences in yields are considered varietal rather than responses to treatment. Only the highly susceptible Rowden was not adequately protected to give the maximum expected yield in comparison with other varieties. The 12.5-gal. rate used in combination with resistant varieties appears about the optimum amount from the standpoints of economy of material and yield response.

The wilt was reduced to a negligible amount by treatment in all varieties except Rowden. Wilt percentages from 3 to 6 are not considered as factors affecting yield with good original stands, such as occurred at Tallassee. The roots of wilt-resistant plants in treated plots were protected sufficiently to permit good growth and production (Fig. 1, B). Root-knot indices in several varieties indicate a high infestation on the treated plots. Observation of the roots shows good protection as far as the fumigant extended. Largest galls were formed on roots extending beyond the limit of the effective range of the gas.

SUMMARY

Markedly increased yields and almost perfect control of cotton wilt and nematodes were obtained by the use of 31 and 37 gallons per acre of Dowfume W-10 as a soil fumigant with both resistant and susceptible varieties of cotton.

More economical control and comparable yields were obtained with applications of the fumigant at the rate of 12.5 gallons per acre applied in the row only. A variety highly susceptible to wilt and nematodes was not adequately protected at the 12.5-gallon rate of application.

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A SIMPLIFIED METHOD OF PURIFYING TOMATO BUSHY–STUNT VIRUS FOR ELECTRON MICROSCOPY[1]

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INTRODUCTION

The application of electron microscopy to virus studies has allowed a new approach to some of the problems of virus identification. Uniformly sized particles, not found in controls, have been isolated by numerous workers from plants known to be infected with tobacco mosaic, tomato bushy-stunt, southern bean mosaic, and a few other virus diseases. These have been highly purified and, because of their intimate association with infectivity, are now considered to be virus particles. They will be so designated throughout this paper. Purified suspensions of particles of the above mentioned viruses have been photographed and each has been found to have a distinct shape and size.

Stanley (4) has mentioned the possibility of using the electron microscope for diagnostic purposes, but this procedure has not yet been generally adopted, because of the difficult purification procedures required.

Sigurgeirsson and Stanley (3) found it unnecessary to preserve the initial concentration of tobacco mosaic virus particles in the plant sap in order to locate them with certainty in the electron micrographs. Because of the rodlike shape and the large natural concentration of these particles, one part of expressed sap from infected plants to 50 parts of water resulted in identifiable specimen fields, and no special procedure appeared necessary for general observation and for non-specific identification of individual particles. Virus particles such as those of tomato bushy-stunt, on the contrary, do not appear as easily distinguishable rods, but rather as tiny spheres which are similar in size to many of the normal plant components. Also, the concentration of these particles in the infected sap is generally much less than that observed for tobacco mosaic, and a 1:50 dilution of the original plant juice scatters them so thinly that they cannot be photographed with certainty.

We are using the tomato bushy-stunt virus, grown in *Datura meteloïdes*[4] for an electron microscopic study of the mechanism of virus multiplication, and it is obviously desirable to subject the infected plant sap to as mild and brief a purification treatment as possible. Previously adopted purification methods have involved precipitation of the virus with ammonium sulphate, or concentration by high speed centrifugation, but we feel that

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[4] We are indebted to Dr. W. M. Stanley for the suggestion that *Datura* is a very satisfactory host for this virus.

these prolonged procedures are liable to remove whatever characteristic signs of virus reproduction might exist in the untreated sap. We have experimented with simplified techniques of partial purification until we are now able to purify the virus sufficiently for electron microscopic observations by means of a fairly gentle procedure requiring only about 25 minutes following the initial extraction of the sap.

PROPAGATION AND PURIFICATION

Seeds of *Datura meteloides* are germinated in sand and are transferred to sandy loam (20 plants in each 8-inch pot). Some of these pots are set aside for controls. The young plants in the others are inoculated when the first true leaves are about one-half inch across by gently rubbing the leaves with cheesecloth saturated with a mixture of one part crushed infected plants to 50 parts of distilled water and one part of fine carborundum. Carborundum is used to puncture the cell walls without injuring or having any toxic effect upon the cells. Plants are washed off with a stream of tap water immediately after inoculation. More than 95 per cent infection is regularly obtained in this manner.

Inoculated and control plants are placed in a specially constructed cabinet with controlled fluorescent lights and constant temperature of about 22° C. Three days after inoculation, numerous virus particles have always been found in the tips of plants thus infected.

The infected leaves and stem are cut and placed in 15-cc. glass centrifuge tubes where they are crushed and the juice pressed out by use of glass rods, the ends of which have been rounded by flaming. The expressed sap is transferred to clean tubes and diluted with an equal volume of distilled water. Heat coagulation (presumably of protoplasmic proteins and chlorophyll) is carried out as rapidly and at as low a temperature as possible. This is done by dipping the tubes containing diluted sap into boiling water and rapidly retracting and rubbing them with the hand to dissipate excess heat. This process is repeated until the first signs of coagulation appear at approximately 49° C. Coagulation is obtained in less than one minute. The tubes are held in the water bath no more than a total of 10 seconds, in contrast to approximately five minutes required when they are continuously immersed in a bath maintained at 50° C.

The coagulum is then sedimented, and loosely packed Celite (a diatomaceous earth) of approximately one-half the volume of remaining liquid is added. This is shaken, and again centrifuged at 3,000 r.p.m. for 3 minutes, after which the liquid is transferred to another clean tube and spinning is repeated for five minutes. Another transfer and spinning for 3 minutes is advantageous in eliminating the diatom particles. Drops of the final clear or slightly yellow-brown liquid are then placed on collodion or Formvar (polyvinal formal) films mounted on the usual electron microscope specimen screens. Each drop is allowed to remain in position over a screen for one-half minute and then as much liquid as possible is with-
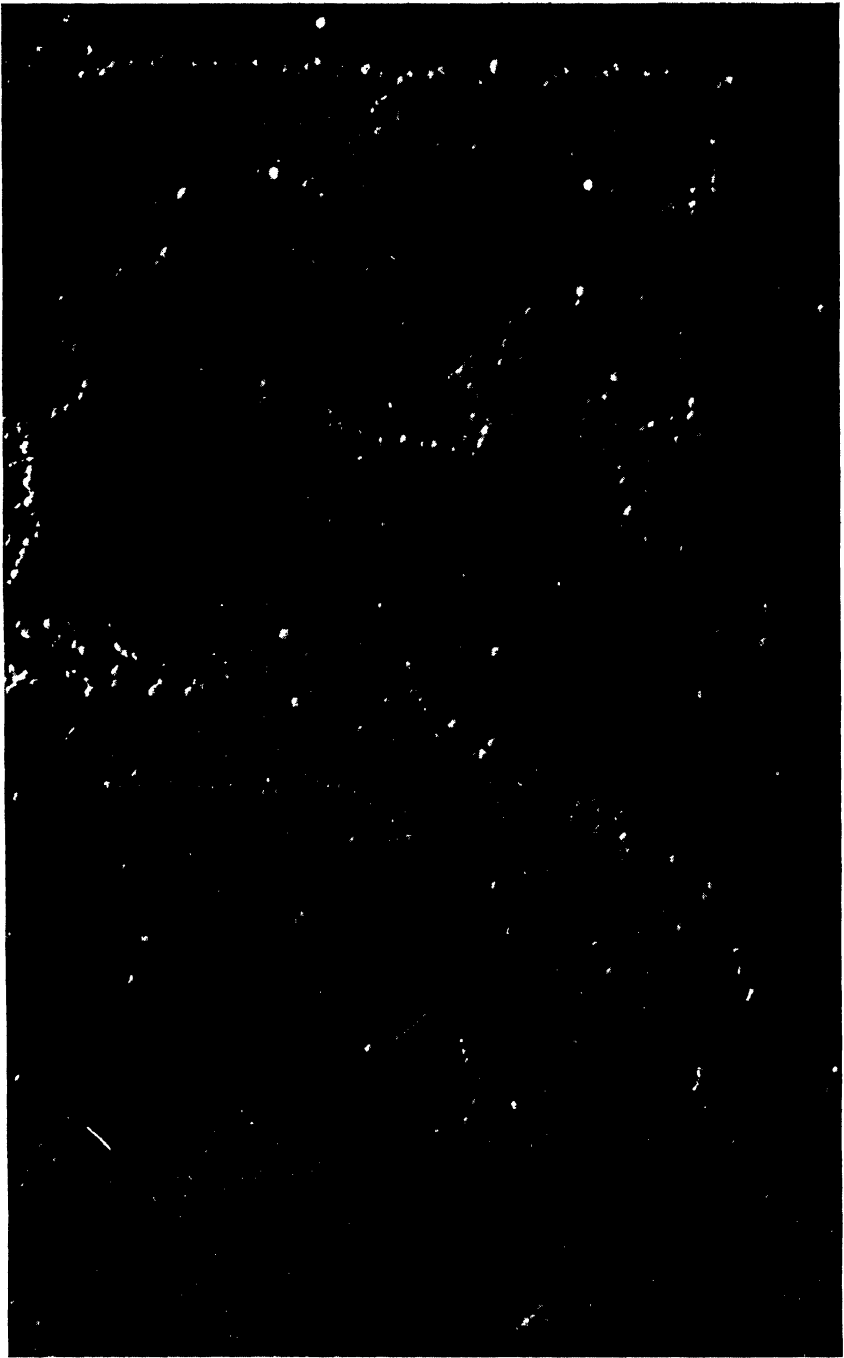
FIG. 1. Electron micrograph of the tomato bushy-stunt virus grown on *Datura meteloides*. Virus suspension allowed to dry on Formvar film after purification, and then shadow-cast with uranium. Ruled line shows one micron. Magnification is thus 45,000 ×.

drawn by a pipette. The remaining liquid is allowed to dry on the film. The screens containing this preparation have been shadow-cast with uranium at a 3-to-1 angle, using the technique of Williams and Wyckoff (5), and have been observed and photographed with an RCA type B Electron Microscope.

As a check, uninfected plants have been submitted to the same procedure as the infected. Whenever particles apparently similar in size to those found in infected material were seen with the electron microscope, they were photographed. In every instance the photographs indicated clearly that there were no spherical particles in the purified control material comparable to those which were always found in preparations from the infected plants.

Datura plants inoculated with the purified liquid, samples of which yield good photographic indication of virus particles, become infected in the usual manner. It would require comparable series of inoculations with successive parallel dilutions of processed and non-processed sap to determine conclusively whether or not the purification technique inactivates part of the virus, but rough experiments so far made do not indicate any loss of infectivity. Bawden (1) has compared the infectivity of unheated and heated virus preparations and has found nearly 50 per cent loss of infectivity with treatment at pH 6 and 50° C. for ten minutes. It is presumed that the short period of heating in the present procedure would have less effect than that observed by Bawden though the temperature of that part of the liquid adjacent to the tube wall may exceed 50°.

We have found it impossible to carry out the purification procedure without the addition of distilled water to the original sap. It is probable that if the normal plant proteins are coagulated from too concentrated a solution (or suspension) they carry down the virus particles with them. This might come about merely by mechanical entanglement with associated particles in a suspension so concentrated that interparticulate freedom of movement is not possible.

Purification by the method described is complete enough to permit the formation of flat layers of aggregates of uniform-sized spherical virus particles when Formvar films are used. A geometrical arrangement is observed in these aggregates (Fig. 1) which might be expected to result from particles of such uniform size that they adjust to that arrangement which utilizes space most economically, i.e. in a square or a hexagonal arrangement as viewed vertically. In some photographs, areas showing as many as three distinct layers have been observed (Fig. 2). When drops of virus suspension purified in the same manner are dried on collodion films, small aggregates among numerous rather evenly spaced particles result (Fig. 3).

Particles small enough to suffer considerable Brownian movement might by such movement adjust themselves to a regular geometrical or crystalline arrangement as they dry out of suspension. Since it may be assumed that
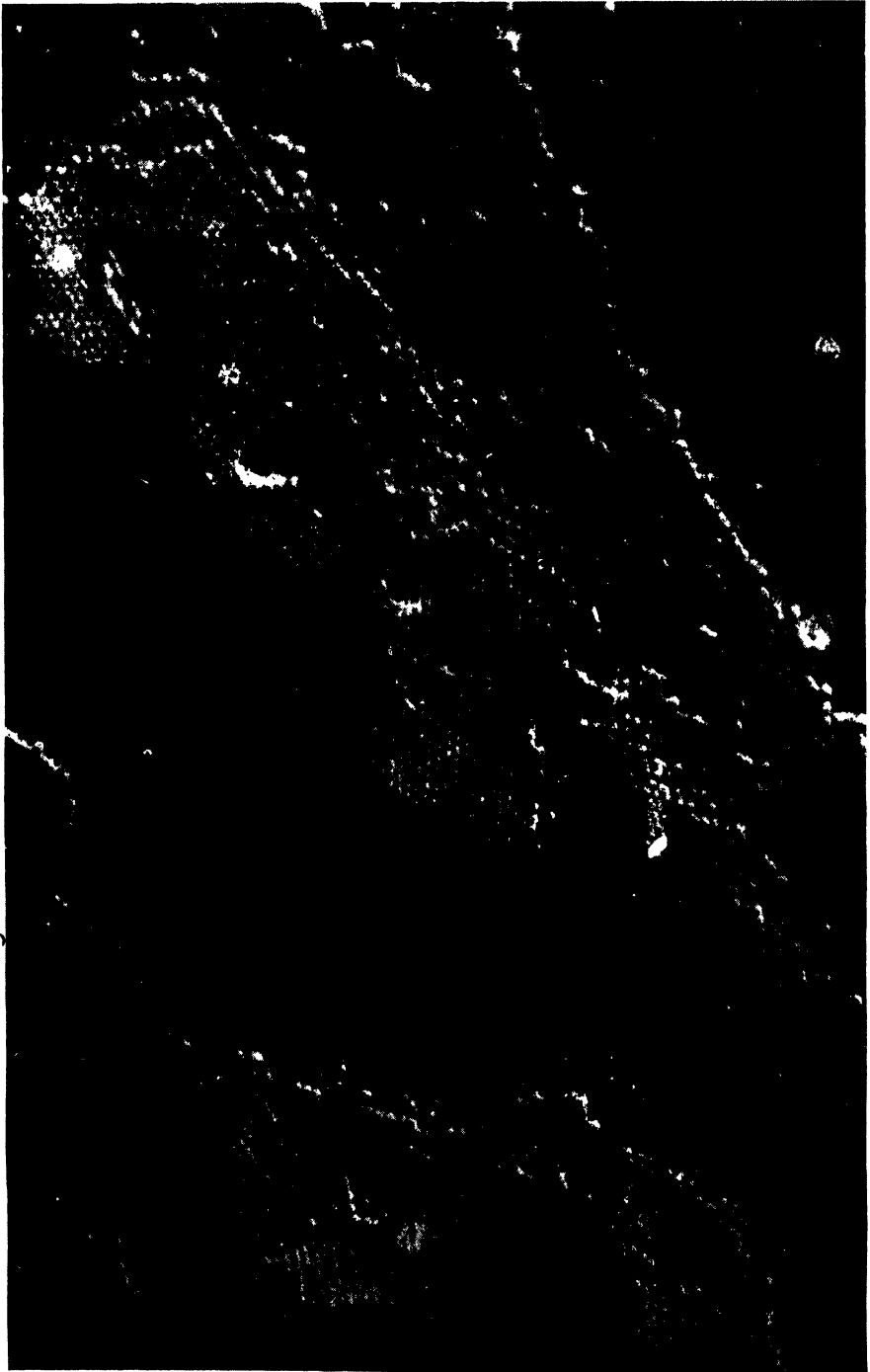
FIG. 2. Same preparation as shown in figure 1. Stratification of aggregates into three distinct layers is plainly visible. Magnification 45,000 ×.
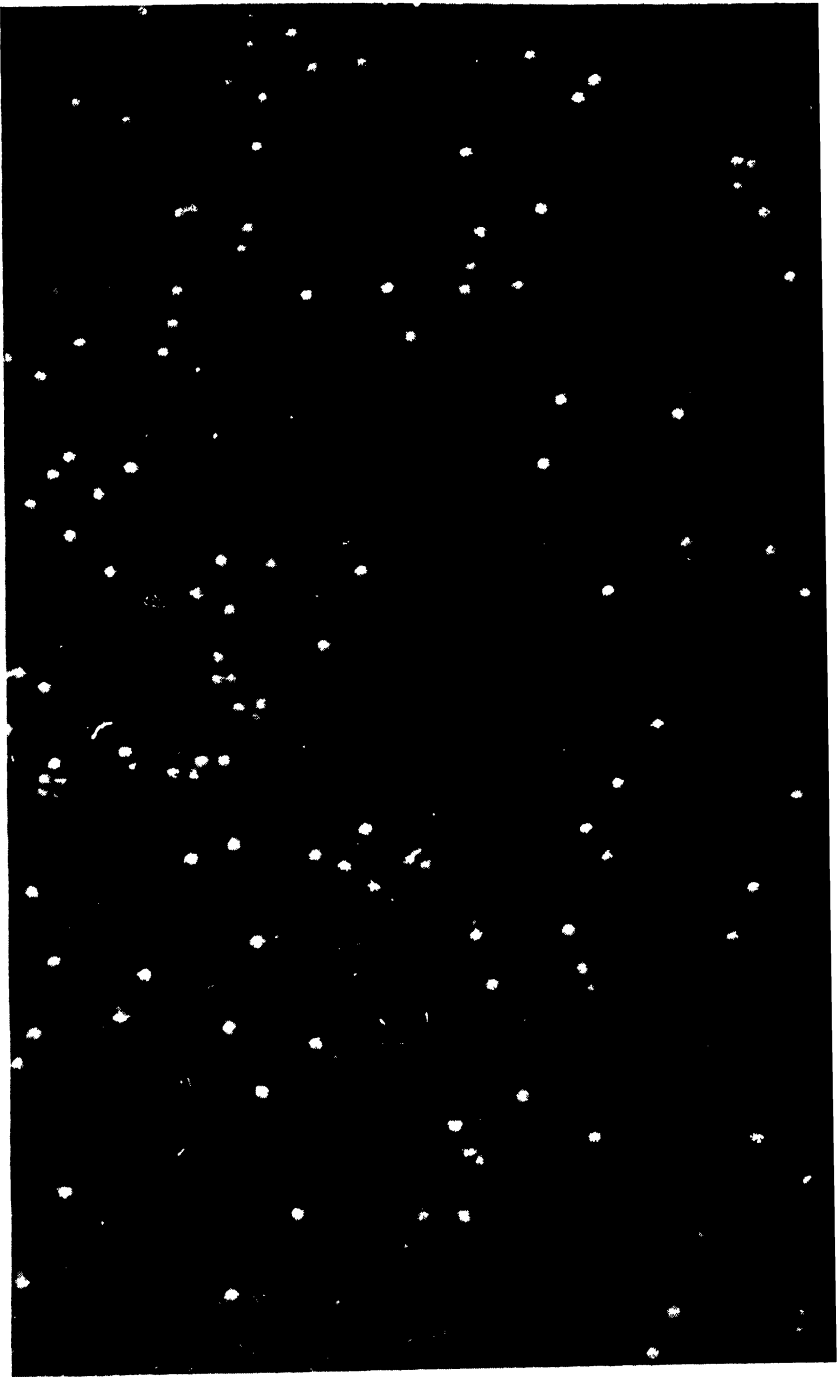
FIG. 3. Preparation similar to that shown in figure 1, but allowed to dry on collodion film. Magnification 50,000 ×.

very slight forces of adsorption or of differential wetting might mask the Brownian motion forces and be sufficient to prevent the orderly arrangement of uniform particles of colloidal dimensions, we need not necessarily assume that the same results should be obtained with surfaces as different in their adsorbing behavior as collodion and Formvar appear to be. The results as indicated above seem to show that such a condition actually exists.

Price, Williams, and Wyckoff (2) have previously recorded crystalline structure of tomato bushy-stunt and southern bean mosaic virus particles following purification by differential ultracentrifugation.

SUMMARY

A new procedure requiring only small amounts of infected plant material has been developed for rapid purification by differential adsorption of ultramicroscopic particles from the sap of plants of *Datura meteloides* infected with tomato bushy-stunt virus preparatory to examination by the electron microscope.

The purification procedure involves: (a) coagulation of normal protoplasmic constituents by heating briefly to approximately 50° C., (b) adsorption of other normal components onto diatomaceous earth, (c) centrifugation at 3,000 r.p.m. at specific intervals to eliminate coagulum and diatom material, and (d) application of suspension to Formvar or collodion films and subsequent removal of excess liquid.

Suspensions thus obtained contain vast numbers of particles of uniform size which are completely lacking in control preparations from uninfected plants and are therefore interpreted as particles of bushy-stunt virus.

Such preparations are actively infective to *Datura*.

When suspensions of virus particles purified by this technique are dried on films of Formvar, electron micrographs reveal regular aggregates of crystalline appearance in which the lines of particles intersect at angles of 90° and 120°.

When the same preparation is dried on collodion, electron micrographs show only small aggregates among numerous rather evenly spaced particles.

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CHEMICAL TREATMENT OF SOYBEAN SEED IN RELATION TO NODULATION BY NODULE BACTERIA[1]

M. F. KERNKAMP

Along with the phenomenal rise of the soybean industry in Minnesota in recent years, problems concerning cultural practices are beginning to appear. One of the most pertinent questions at the present time is whether chemical seed treatment will kill nodule bacteria (*Rhizobium leguminosarum*) and thus nullify the beneficial effects of using a commercial nitrogen inoculant.

Preliminary results reported by Allington, Kent, Tervet, and Koehler (2) indicated that nodulation on soybean roots was slightly reduced by seed treatment in Alabama, but was not impaired in other areas.

Kadow, Allison, and Anderson (3) reported a reduction of nodulation when bacterial inoculant was applied to pea seed after treatment with Semesan, cuprous oxide, and zinc oxide, and sown in partially sterilized soil. They found no reduction in nodulation when similarly treated seed was planted in field soil where the nodule forming bacteria were already present. Likewise, Sharvelle, Young, and Shema (5) found no harmful effect of Spergon on nodule bacteria on peas in field tests.

Miller (4) found that Spergon had no effect on root nodulation in peanuts grown in the greenhouse either when seed was inoculated with commercial preparations of nodule bacteria or left uninoculated. Albrecht (1) compared yields of peanuts grown from nontreated and Spergon treated seed in plots that had been inoculated with nitrogen bacteria. His results indicated that Spergon was not injurious to nodulation.

The experiments reported here were designed to test in more detail the effects of seed treatments on nodulation in soybeans when chemical seed disinfectants and nodule bacteria were both applied to seed at different times or at the same time.

PROCEDURE

The following methods of seed treatment and inoculation were tested in the greenhouse: (1) seed treated with Spergon and not inoculated; (2) seed treated with Spergon 2 days after inoculation; (3) seed treated with Spergon and inoculated at the same time; (4) seed treated with Spergon and inoculated 2 days later; (5) seed inoculated but not treated with Spergon; and (6) seed not treated or inoculated. Each treatment was replicated three times and each experiment was repeated three times. One replicate comprised 10 plants in a 6-inch plot, thus giving 30 plants per treatment per experiment. The greenhouse tests were made with soil auto-

claved for 4 hours, and with non-autoclaved soil, both experiments being run simultaneously.

Spergon (tetrachloro-para benzoquinone) was applied at the rate of 2 oz. per bu., and the inoculant, commercial Nitragin, was applied according to the recommended wet method. In all tests attempts were made to duplicate farm procedure insofar as possible.

The variety Habaro (Minn. No. 109) was used throughout. The number of nodules on the roots of plants were counted after they had grown for approximately 6 weeks. By that time the plants were approximately 18 inches high, and the soil was thoroughly penetrated by roots. The data were analyzed statistically by analysis of variance.

Preliminary results of a field experiment also are presented. In this test the plan was identical to that given above, except that New Improved Ceresan (ethyl mercury phosphate) and Semesan Jr. (ethyl mercury phosphate) were also tested on the varieties Ottawa Mandarin (Minn. Acc. No. 219) and Wisconsin Manchu No. 3 (Minn. Acc. No. 210).

RESULTS

Table 1 summarizes three greenhouse experiments in which soybean

TABLE 1.—*Summary of greenhouse experiments on the number of nodules on Habaro soybeans grown from seed inoculated with Nitragin bacteria, treated with Spergon, and planted in non-autoclaved soil*

| Experiment and Date | Treatment and number of nodules[a] | | | | | |
| | Spergon | | | | No Spergon | |
	No Nitragin	Nitragin 2 days before Spergon	Nitragin with Spergon	Nitragin 2 days after Spergon	Nitragin	No Nitragin
1. April, 1947	55.7	46.6	62.3	46.3	28.6	52.0
2. June, 1947	234.0	283.0	237.3	322.7	274.6	334.0
3. March, 1948	15.3	56.6	39.0	66.0	72.0	16.3
Mean of all experiments[b]	101.7	128.7	112.7	145.0	134.4	125.1

a Each figure represents the average number of nodules on three replicates of 10 plants each.
b No significant difference between means when analyzed by analysis of variance.

seed was treated with Spergon, inoculated with Nitragin, and planted in non-autoclaved soil in the greenhouse. Nodules developed equally well regardless of whether or not inoculum was applied, and regardless of the time of application of inoculant and Spergon. The means of all three experiments varied only from 101.7 to 145.0 nodules per 10 plants for all treatments. Although there seemed to be a tendency for more nodulation when the inoculum was applied after treatment with Spergon, the differences between means were not statistically significant. It is assumed that nodule forming bacteria were already present in the soil prior to seed in-

oculation.   These results are in agreement with those of Kadow et al (3), who reported that if pea seed was planted in soil already containing nodule bacteria, nodules developed regardless of inoculation or treatment with a chemical disinfectant.

There was considerable difference between experiments in degree of nodulation.   The low number of nodules in the first experiment (Table 1) can be explained by the fact that the plants were given less water than is optimum for nodule formation.   The discrepancies between the other experiments could be explained by the fact that different sources of soil and inoculum were used in the different experiments.   The important thing is that the trend was the same in each experiment, and there was no significant difference between the over-all means.

Parallel experiments were made, along with those described, using par-

TABLE 2.—*Summary of greenhouse experiments on the number of nodules on Habaro soybeans grown from seed inoculated with Nitragin bacteria and treated with Spergon and planted in soil autoclaved for 4 hours*

Experiment and Date	Treatment and number of nodules[a]					
	Spergon				No Spergon	
	No Nitragin	Nitragin 2 days before Spergon	Nitragin with Spergon	Nitragin 2 days after Spergon	Nitragin	No Nitragin
1. April, 1947	1.0	6.3	5.0	36.3	4.3	1.0
2. June, 1947	23.7	47.6	83.7	67.3	491.0	15.7
3. March, 1948	201.7	269.3	299.7	275.6	338.3	82.0
Mean of all experiments[b]	75.6	107.7	129.4	126.4	277.9	32.9

[a] Each figure represents the average number of nodules on three replicates of 10 plants each.
[b] See table 3 for significant differences when analyzed by analysis of variance.

tially sterilized soil, autoclaved 4 hours.   The results are presented in table 2, and the statistical significance of the data is presented in table 3.

As in the experiments with non-autoclaved soil, there was no statistically significant difference in nodulation when Spergon without inoculum was compared to Spergon with inoculum applied at different times.   But when no inoculum and no Spergon were applied, there was a significant reduction of nodulation compared to inoculation at the same time as treatment and inoculation after treatment.   When seed was inoculated without Spergon, nodulation was greatly increased over all other combinations of inoculation and application of chemical.

These results again indicate that Spergon used in combination with Nitragin, even in partially sterilized soil, did not significantly influence nodulation in soybeans.   Nodulation was significantly increased, however, when Nitragin was applied without Spergon as compared to Nitragin with Spergon in any combination.

Here again differences will be noted between the data from different experiments. The reasons given for such variation in the experiments with non-autoclaved soil will apply here since they were made at the same time.

An experiment was made in the summer of 1947 to test the same methods of inoculation and seed treatment on nodulation and yield in the field. In addition to testing Spergon with inoculant, Semesan Jr. and New Improved Ceresan were tested. Also, two additional varieties, Ottawa Mandarin and Wisconsin Manchu No. 3, were included in the test. The experiment was laid out in randomized blocks, replicated four times. Nodules were counted on an average of approximately 15 plants per row on August 25, 1947, and yields were recorded at harvest time. The plot was planted on a piece of ground that had never been known to be planted to soybeans previously.

The results are based on only one year's data and are necessarily preliminary. There was no statistically significant difference in nodulation or yield regardless of what combination of inoculum and either of the three seed disinfectants was used.

TABLE 3.—*Significance[a] of data on nodulation given in table 2*

Spergon, no Nitragin	vs. Nitragin 2 days before Spergon	not significant
do.	vs. Nitragin with Spergon	do.
do.	vs. Nitragin 2 days after Spergon	do.
No Spergon, no Nitragin	vs. Nitragin with Spergon	significant
do.	vs. Nitragin 2 days after Spergon	do.
Nitragin, no Spergon	vs. All Others	do.

[a] Significance was determined by analysis of variance and comparing means, using L.S.D. = 90.1 at the 5 per cent level.

These results agree with the results in non-autoclaved soil, and they agree with reports of Kadow et al (3) and Miller (4), who both stated that nodulation is not affected by seed treatment or inoculation when seed is planted in soil that has not been partially sterilized or in soil where the nodule bacteria are already present.

DISCUSSION AND CONCLUSIONS

The application of Spergon with commercial Nitragin did not influence nodulation when the seed was planted in soil that already contained the bacteria; and preliminary results from one field experiment indicate that seed treatment with Spergon, New Improved Ceresan, or Semesan Jr. had no effect on nodulation or yield. Even in soil autoclaved for 4 hours the various combinations of Spergon with or without commercial Nitragin did not affect nodulation. However, results with steamed soil indicated that Spergon reduced nodulation as compared with the non-treated check. The latter may be interpreted to mean that Spergon may reduce nodulation when inoculated seed is planted in soil that contains a small number of nodule bacteria.

Only soil from the Experiment Station farm at St. Paul was used in

the tests, and the results obtained may not apply to other soil throughout the State or the soybean area of the Corn Belt. But with the widespread production of soybeans, it seems likely that nodule bacteria are also widespread, and therefore Spergon would not reduce nodulation in most soybean fields. This is supported by the fact that there were nodules on plants in one field that had never been known to have grown soybeans previously, and is further supported by results of other experimenters (1, 2, 3, 4, 5), who found no apparent deleterious effect of seed disinfectants when legume crops were planted in soil already containing the bacteria.

The time of application of inoculant or fungicide does not appear to be of major importance, since the degree of nodulation did not differ significantly with different times of application. However, in view of the fact that slightly more nodulation resulted when the inoculant was applied after the Spergon, even though the differences were not statistically significant, it is recommended that that method be followed. The directions that appear on the containers of the Spergon and inoculant appear to be adequate.

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BACTERIA IN THE STORAGE ORGANS OF HEALTHY PLANTS

IAN W. TERVET[1] AND JOHN P. HOLLIS[2, 3]

INTRODUCTION

It is generally believed that healthy plant tissue is free from micro-organisms. This viewpoint draws most support from negative results obtained in the last century (2, 5, 15). A complete review of earlier work is furnished by Berthold (1). Evidence to the contrary is of comparatively recent origin (3, 6, 7, 9, 10, 11, 12, 13, 14). The most pertinent reports have all arisen since 1936 and are mostly independent of one another. Since this work is also of independent origin, no formal review of literature will be given.

The stimulus for the investigation reported herein arose from a chance discovery by the senior author of bacterial action in cultures of potato plugs submerged in sterile water. Breakdown of the plugs occurred and with this the liberation of whole starch which was deposited in the bottom of the culture tubes. Since the plugs of tissue had been removed aseptically, it was considered probable that the bacteria might be present inside the tubers. Experiments were conducted during the summer of 1947 to test this possibility.

MATERIALS AND METHODS

Tubers and storage organs of potato, carrot, turnip, red beet, and sweet potato along with fruits of tomato and kohlrabi buds were tested for the internal presence of bacteria. The organs were washed in tap water, placed in either 1:1000 acid mercuric chloride or calcium hypochlorite of 2 per cent chlorine strength for 5–20 minutes, removed, and blotted so as to remove excess moisture.

Plugs of tissue were removed by two methods. The first utilized a cork borer that was dipped in alcohol and flamed. A flap of tissue was torn from the surface of the organ to be tested, the borer inserted and pushed through to the opposite side. The surface tissue at the end of the borer was then extruded by means of a sterile glass rod and excised with a sterile scalpel. The plug of tissue was then pushed out into a sterile culture tube water blank. The second method utilized a scoop commonly employed in transporting chemicals for weighing. The tuber was cut part way, then broken into two halves. The scoop previously flamed red hot, cooled in 95 per cent alcohol and reflamed, was then inserted into the broken surface and a wedge-shaped piece of tissue removed. Figure 1, A shows plugs

taken by the two methods.   Cork borers used in the test were always auto-
claved before the next lot was sampled.

The method of sampling must be noted.   Tubers of freshly harvested
Red Warba potato comprising 12 lots taken from Central Nebraska in
August, 1947, were selected for large size and 5 plugs taken from each
tuber transectionally.   Likewise, 5 plugs were taken from kohlrabi buds,
red beet, turnip, and carrot, and 3 plugs from green tomatoes.   No par-
ticular pattern of plug removal was used in sampling other lots.

All cultures were incubated at room temperature.   Over the period
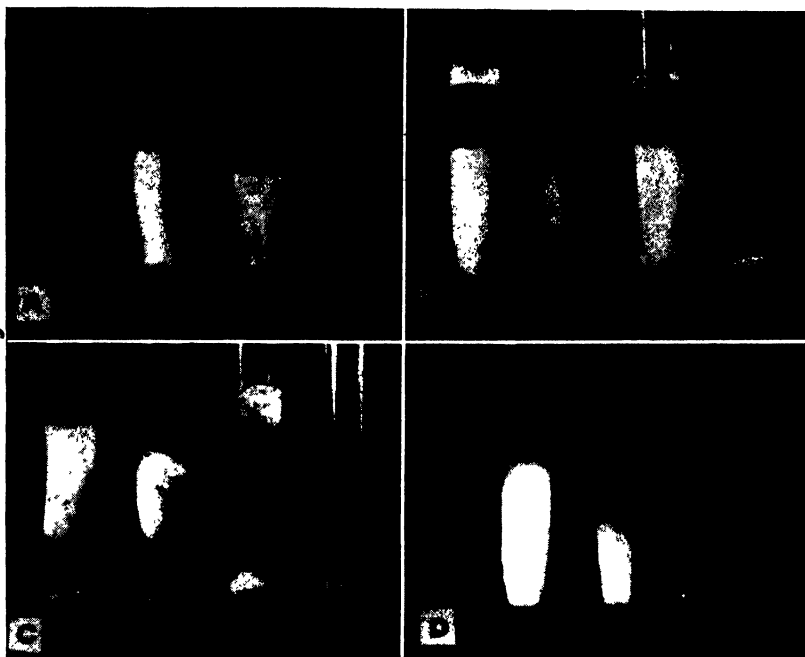
FIG. 1.   A. The two types of aseptic potato plugs used in determining the occur-
rence of bacteria in plant tissue.   B. Types of bacterial growth or tissue change found
in aseptically produced potato plugs 1. to r. turbidity, pellicle, green fluorescence, tissue
breakdown.   C. Stages in breakdown of potato plugs resulting in starch liberation.
Left: plug in initial stages of breakdown.   Center tubes: further stages in breakdown.
Right: tissue digested leaving deposit of starch at bottom of tube.   D. Three steps in
the blackening of submerged potato plugs.

during which the work was performed, the average laboratory temperature
was in excess of 30° C.

EXPERIMENTAL WORK

In the initial phases of this study it was noted that complete breakdown
of "sterile" potato tissue submerged in sterile water with the liberation of
whole starch grains from the cells was of rather infrequent occurrence.
The most prevalent type of growth in submerged plug cultures was a uni-
form turbidity with gas formation.   Turbidity with green fluorescence
was less frequent but occurred with more regularity than complete break-

down.   There was high correlation between certain lots of potatoes sampled in early tests and the presence of bacteria producing turbidity and gas.   No such correlation existed between potato lots and the presence of organisms responsible for complete breakdown of potato tissue.

In order to learn more about the prevalence and distribution of bacteria in healthy potatoes, a number of experiments were conducted in sequence.   Table 1 gives the results of these tests.   The number of cultures

TABLE 1.—*Summary of bacterial isolation tests from healthy plant tissues using plugs of tissue removed aseptically and submerged in sterile water*

Plant and plant part	Lot No.	Method	Incubation time in days	Number of plugs	Sterile plugs	Gas and turbidity	Gas and green fluorescence	Pellicle	Cellulose decomposition
Potato Tuber[a]	1–12	Borer	10	600	435	78	16	68	0
Do	13	Do	15	30	20	10	0	0	0
Do	13	Scoop	2	10	1	7	0	0	2
Do	13	Do	7	30	2	28	0	0	0
Do	13	Do	8	34	4	27	4	1	0
Do	13	Do	2	30	27	2	0	1	0
Do	13	Do	15	60	45	15	0	0	0
Do	14	Borer	4	24	1	0	0	0	23
Do	15	Do	3	70	38	26	0	6	0
Aerial tuber	16	Do	3	15	8	0	0	3	4
Do	16	Do	4	85	50	18	0	14	3
Do	16	Do	8	150	136	14	0	0	0
Kohlrabi Bud	1	Do	8	50	-36	13	0	1	0
Red Beet Root	1	Do	8	50	30	20	0	0	0
Turnip Root	1	Do	8	50	9	41	0	0	0
Carrot Root	1	Do	8	49	34	15	0	0	0
Sweet potato Root	1	Scoop	7	30	30	0	0	0	0
Tomato Fruit	1	Borer	8	34	34	0	0	0	0

[a] Red Warba variety.

with turbidity and gas formation far exceeds the number with any other type of growth.   Microscopic examination of turbid cultures revealed the presence of Gram negative rods although other types of bacteria frequently were present.   Indeed, many cultures with initial turbidity were later found to possess a pellicle.

These cultures were always grouped with other pellicle-forming cultures and are enumerated this way in table 1.   Figure 1, B shows the cultural types encountered.   The few cultures in which there was complete

breakdown with starch liberation in this series of tests (Fig. 1, C) suggest that either the presence of these bacteria varies widely among lots of potatoes or that superficial contamination is responsible for the erratic results. It is significant that not one culture of this type was encountered in the large experiment involving 12 lots of potatoes. In this test the percentage of cultures in which growth occurred varied between 8 and 54 per cent for the various lots. Although 5 plugs were taken from each tuber there was no tendency for the bacteria to be distributed in tuber units. The converse was true in the case of carrots. No adequate explanation can be given of these results at present.

In the blackening of potato plugs in submerged culture, due to formation of melanin pigment (Fig. 1, D), there was complete agreement between tuber unit and the extent and intensity of color formation. The intensity and extent of blackening increased with the time beginning at the top and progressing toward the base. Some sets of 5 plugs remained completely white over the entire period of incubation.

Of other plant tissues from which isolations were made, turnip appears to be the most uniformly infested with bacteria. An examination of 10 cultures taken at random from tubes with growth in the turnip series revealed the presence of Gram negative rods in all 10 cultures. In addition to Gram negative rods, Gram positive rods of different size were found in one tube.

The industrial implication of cellulose breakdown with starch liberation made it desirable to conduct a preliminary investigation as to the nature of the organism bringing about this action. Microscopic examination of raw potato plug cultures in which breakdown was complete and starch was liberated revealed the presence of several morphologic types of bacteria. Uniformly present were large Gram positive rods, medium-size Gram positive rods, small Gram negative rods, and large clostridial forms.

It was possible to obtain pure cultures of the clostridial form by means of two successive heat treatments of aseptic potato plug cultures freshly inoculated with liquid from a broken-down culture. When these tubes were treated in water at 80° C. for one-half hour, the clostridial form survived this treatment and produced gas and turbidity after 24 hours. There was no breakdown by this organism alone. Morphologically these clostridial rods are variable. The ellipsoidal spores are borne terminally and are Gram positive. The vegetative part of the rod stains a light violet color with gentian violet. This part or "tail" is variable in length and diameter and may or may not be present with the spore.

The inability of pure culture transfers of this organism to raw potato plug cultures to bring about breakdown suggested that a complex of two or three organisms might be responsible for this action. Further tests were devised using potato-dextrose agar adjusted to a reaction of pH 7.0 as the test medium. Dilutions or loops of the original breakdown liquid

were used for all initial transfers to potato-dextrose agar. There was no growth in either potato-plug or Petri-dish cultures incubated in pure nitrogen indicating that at least one of the essential organisms was not a strict anaerobe. Inoculation of slants under sterile mineral oil, with liquid from a plug culture which had broken down, resulted in a whitish growth composed of the clostridial rod and small Gram negative rods which tended to be associated end to end in pairs. When a loopful of this growth was transferred to fresh, raw plug-cultures complete breakdown with starch liberation occurred in 48 hours at temperatures above 30° C. The optimum temperature for breakdown under the conditions imposed proved to be about 35° C.

Pure cultures of the Gram negative rod were obtained from slants by transferring the inoculum to dilution plates, which were then covered with a layer of sterile mineral oil, or by incubating the dilution plates under an oxygen tension of 10 mm./Hg. maintained by continuous faucet suction. Apparently the clostridial form required certain growth factors which were not carried over in sufficient quantities to dilution plates. It was noted also that successive transfer of the two organisms to fresh slants under oil resulted in loss of breakdown activity when these daughter slants were used for inoculating raw plug cultures. Obviously, the age of the slant culture may be important in loss of activity on potato-dextrose agar.

When the Gram negative rod was transferred to raw plug cultures, turbidity with gas formation resulted. The combination of both organisms was found necessary for breakdown. The complex was best retained in liquid from a culture which had previously broken down and at the time the work was terminated had retained viability in a sample of this liquid for about 5 months.

The identity of these organisms has not been pursued further. Muratova (8) obtained raw starch liberation from potatoes with the aid of an organism closely resembling *Bacillus felsineus* Carbone. Paziuk (10) described a single pleomorphic plectridial form which he showed capable of liberating raw starch from potatoes. It is not clear whether he was working with only one organism, because one type of rod could be seen in surface colonies while another type, a spore-forming plectridium, was found between the agar and glass at the base of the same culture. Buromski and Matiushenko (3) report breakdown of potatoes with starch liberation as due to a combination of cocci and rods.

The Gram negative rod reported in this work formed small, glistening white colonies 0.5–5 mm. in diameter. Most surface colonies were 2 mm. in diameter after 48 hours. The clostridial form reported herein is similar morphologically to the organism described by Muratova (8).

Further tests were conducted by inoculating the breakdown liquid from potato plug cultures into submerged tissue cultures of cooked potato, sweet potato, carrot, and alfalfa leaves and petioles. Cooked potato plugs broke

down completely with starch also disappearing. Breakdown in carrot and alfalfa occurred as with raw potato plugs. Sweet potato gave unique results in that cell separation occurred with no breakdown in individual cell walls.

<div align="center">DISCUSSION</div>

From the standpoint of plant pathology, the most significant of these results is that pointing to the possible presence of a mixed bacterial flora in healthy storage organs. While bacterial types have not been identified, it is obvious that they may represent common soil forms. Sanford (12) cut thin sections from the stele in the stems of potatoes and beans from which he isolated a mixed bacterial flora. Taproots of sweet clover and alfalfa likewise yielded bacteria. He also isolated bacteria from the xylem of large potato tubers.

The method of isolation used in these tests was not sufficiently sensitive to detect the presence of bacteria in all healthy material tested; indeed, the majority of separate units yielded no bacteria. This indicates that the bacteria are present in small numbers.

Two principal types of entry portals are suggested by this and previous work. Sanford's results indicate that the bacteria are present in the vascular system of plants. This would suggest that bacteria may enter through natural wounds caused by emergence of secondary roots and that then they could be translocated to upper parts of the plant. Bacteria gaining entrance in this way should be present only in small numbers and their translocation into tubers then is a function perhaps of unusual external conditions. Bacteria may enter directly into storage organs through natural openings such as lenticels or through wounds and perhaps even through such protective barriers as periderm. If the latter hypothesis is true, we would expect to isolate bacteria more readily from surface layers than from stelar portions of storage organs and roots. Davidson (4) recently showed that bacteria of a soft-rot type gain entry through the stem end and eyes of the tuber and proceed along the pith strands into the tuber when these tubers are submerged in water for periods up to 5 days. Conceivably bacteria could enter tubers in this fashion prior to digging and effect no change in their healthy appearance.

As early as 1897, Zinsser (15) injected common species of bacteria into stems, roots, and leaves of various plants. He found that survival of certain forms occurred over a significant period but there was no multiplication of bacteria and very little movement from the point of inoculation. Berthold (1) found that bacteria and fungi would penetrate into vessels of a woody stem as a result of water intake from a cut surface. He found that survival of bacteria injected into healthy tissues occurred for as long as 10 months but no reproduction of adapted bacteria in the tissues was observed. Buromski and Matiushenko (3) as a result of working with starch-liberating bacteria of potatoes concluded that cocci and rods which

they found present in potato tubers could only develop intensively if the cells were ruptured by freezing or asphyxiated by submerging in water, both of which would result in death of the cells. Similarly, Romwalter and Király (11) concluded as a result of their isolations of yeast from gooseberries and grapes that a high carbon dioxide concentration promoted fermentation by yeast in healthy fruits, a process which did not occur if the fruits were maintained in air. They observed that, after fermentation of fruit in closed containers had run its course, the tissues were dead. This killing of cells was believed due to the presence of anaerobic bacteria. After restoration to air of berries in closed containers, the aerobic bacteria became active. It was considered that these were present inside the fruits and that conditions were made favorable for their growth by the previous decomposing activities of anaerobes.

These results show that furtherance of knowledge as to the occurrence and role of bacteria in healthy plant tissue may depend upon unusual methods of isolation. Obviously, bacteria can be isolated by bringing the organisms into contact with favorable nutrient substrates. The results above indicate the necessity, in some cases at least, of altering the natural substrate so that bacteria present in small numbers may develop in situ.

SUMMARY

1. Evidence is presented, from isolation studies involving more than 1000 submerged plug cultures, which points to the possible presence of bacteria in healthy storage organs of potato, red beet, turnip, carrot, and kohlrabi.

2. The results suggest that a mixed bacterial flora may be present.

3. Two morphologic types of bacteria comprising a clostridial form and a Gram negative rod were found to be responsible for breakdown of potato, carrot, and alfalfa tissue with liberation of cell contents, principally raw starch.

4. The two organisms were separated by means of heat treatments and dilution and grown in pure culture.

5. Blackening of potato plugs due to formation of melanin pigments occurred in characteristic fashion in individual tuber units.

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MICROASCUS TRIGONOSPORUS FROM CEREAL AND LEGUME SEEDS

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AND J. G. DICKSON

During the course of studies on seed-borne fungi in barley, oats, wheat, and soybeans, isolations of a species of *Microascus* which resembled very closely *M. trigonosporus* Emmons and Dodge (2) were secured in great frequency. Since the seeds were surface sterilized before plating, it was of interest to study whether the *Microascus* species was of the nature of a pathogen. A review of literature indicated that two records of the occurrence of *Microascus* species had been reported in the United States: *M. intermedius* Emmons and Dodge on diseased strawberry roots in North Carolina (2) and *M. lunasporus* Jones isolated from the dermal infections on the human hand (3). The latter was similar to *M. trigonosporus* Emmons and Dodge (2) also secured from the dermal infections of the hand of a man in Puerto Rico. These reports of possible pathogenic nature of the fungus led the writers to study the isolates from barley, oats, wheat, and soybeans in some detail.

MATERIALS AND METHODS

The seed samples used in the present studies were collected in different localities in the United States. Some of these samples were a few months from harvest and others were as old as twenty-five years. The seed samples were divided down to approximately 100 kernels in each lot and were surface sterilized by suspending in coarse cotton cloth and dipping into a solution of 1 part 95 per cent ethyl alcohol and 2 parts Chlorox solution (5.25 per cent sodium hypochlorite). The treatment period in the hypochlorite solution was one minute for barley, soybeans, and wheat, and 30 seconds for oats. The surface sterilized seeds were plated on potato-dextrose agar at the rate of ten seeds per Petri dish and incubated at 20° C. for 4–6 days. Observations for the developmental stages of conidia and perithecia were made at various intervals.

ISOLATION AND MICROSCOPIC STUDY OF THE FUNGUS

The first series of isolates of *Microascus* were made from "Mammoth yellow" soybeans grown in Alabama and stored longer than six months. The grayish-white colonies of the *Microascus* developed around the surface-sterilized seeds. In a similar manner barley, oats, and wheat kernels yielded the characteristic colonies of the *Microascus* when they were surface sterilized and plated on potato-dextrose agar (Fig. 2, A, B). In one sample of barley that had been stored in bottles in the laboratory longer than 25 years, profuse development of the *Microascus* colonies was noticed
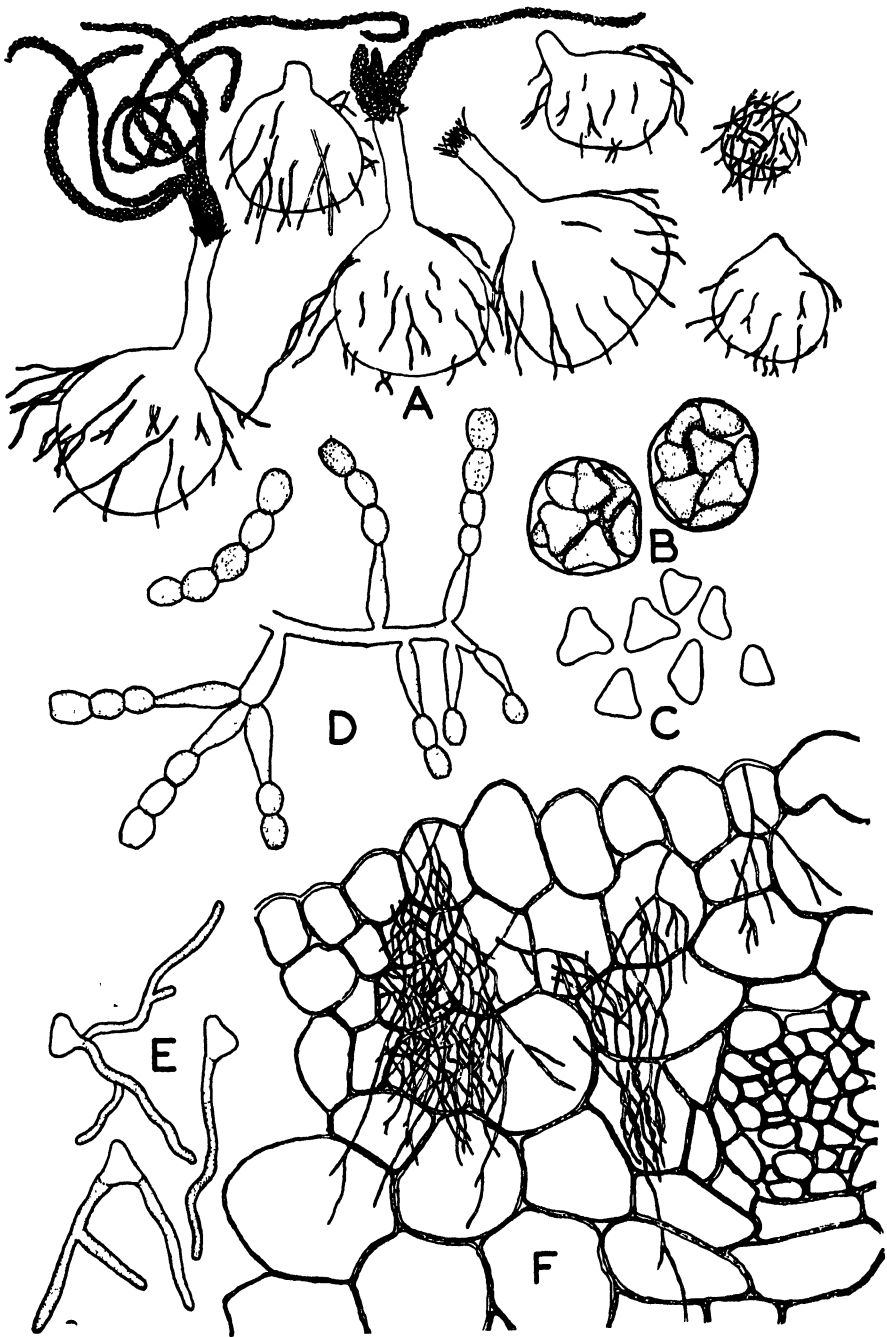
FIG. 1. Mycelium, conidia, and perithecia of *Microascus trigonosporus*. A. Perithecia of different maturity, some with ascospore tendrils (×300). B. Globular asci (×2000). C. Ascospores (×2000). D. Conidiophores and chains of conidia (×1500). E. Germinating ascospores (×1500). F. Mycelium within host tissue (×1320).

on all kernels (Fig. 2, A). The *Microascus* has been isolated from seeds secured from the following places in the United States:

Soybeans (Mammoth yellow variety)—Alabama.

Barley—Brookings, South Dakota; Fargo, North Dakota; East Lansing, Michigan; Mt. Morris and Urbana, Illinois; Crookston, Waseca, and St. Paul, Minnesota; Ames and Kanawah, Iowa; Madison, Wisconsin.

Oats (Vicland)—Madison, Wisconsin; Thermopolis, Wyoming.

Wheat—Madison, Wisconsin.

The fungus apparently is distributed widely in the United States.

The fungus on potato-dextrose agar first formed dull, white colonies composed of fine and closely interwoven hyphae that imparted a bacterioid consistency to colonies. As development proceeded, hyphal branches grew erect and developed conidiophores and catenate conidia characteristic of *Scopulariopsis* Bain., which has been shown by previous investigators (1, 2, 3) to be the conidial stage of *Microascus*. •

The several isolates from barley, wheat, oats, and soybeans were compared morphologically. They were found similar and it was evident that they belonged to the same species. The life cycle of the fungus was studied in culture. The conidial stage developed first with conidia produced on aerial hyphae. Conidiophores were simple or branched and bore at the tip a single sterigma or verticil of sterigmata (Fig. 1, D). Conidia were formed in catenulations that either separated early or remained firmly united for a long time. Spores were ovate to lemon-shaped, smooth, and measured 2.5–3.3×4–$5.5\ \mu$.

Perithecia formed soon after development of conidia, appearing first as scattered, minute black specks. As perithecia developed in large numbers, the colonies turned black. The early stages of the development of the ascocarp have been given in detail by Emmons and Dodge (2) and Jones (3). Young perithecia were yellowish-brown with lax hyphae enveloping them; as they matured they turned carbonaceous black. The ostiole was well developed (Fig. 1, A) and the ostiolar beak varied from a mere protuberance to an elongated neck and resembled those figured by Curzi (1) for *Microascus cirrosus* Curzi. Arrangement of the asci within perithecia was characteristic of the Plectascales such as those occurring in the genera *Penicillium*, *Aspergillus*, and *Thielavia* as mentioned by Emmons and Dodge (2) and Curzi (1). The mature ascus was globose to spherical, with eight triangular ascospores (Fig. 1, B) that were released when the wall of the ascus deliquesced. Mature ascospores were triangular to slightly crescentric (Fig. 1, C; and Fig. 2, C), reddish brown, 3.0–3.5×4.5–$5.2\ \mu$. They were discharged in long cirri bound together by a gelatinous matrix as in some of the other species. In old drying cultures, some of these spore tendrils were several millimeters long and very conspicuous (Fig. 1, A). The shape of the ascospores has been over-emphasized in some of the species

FIG. 2. *Microascus trigonosporus* from barley and oats. A. Platings of 25-year-old barley seed with colonies of *Microascus* from the seeds (Natural size). B. Platings of freshly harvested and naturally infected oats (Natural size). C. Photomicrograph of asci and ascospores (About 750 ×).

described.   While the elliptic ascospores of *M. sordidus* are distinct from the triangular spores of *M. trigonosporus,* there is very little difference between the lunate spores of *M. lunasporus* and *M. cirrosus.*   Since the *Microascus* species under study resembled *M. trigonosporus,* in most of the essential characters, it is proposed to refer the isolates described here to that species.   *M. trigonosporus* has been known so far only from the type locality (Puerto Rico) where it was isolated from dermal infection of man.

THE FUNGUS IN RELATION TO HOST TISSUE

Repeated isolation of mycelium of the *Microascus* from surface sterilized seeds, indicated that the fungus was within the seed tissues.   Studies were undertaken to determine the location of the fungus in the tissue and its early development.   Seed externally infected with the fungus showed no evidences of disease symptoms, either in the dormant state or during germination.   The staining methods used to locate dormant mycelium within tissues of dry seeds were unsuccessful.   Kernels, surface sterilized and plated as described previously, were sectioned and stained with cotton-blue lactophenol after 3 days' incubation at 20° C.   Hyphae were located in the tissues of floral bracts and pericarp.   In the pericarp, hyphae were concentrated as interwoven masses three to four cell layers beneath the epidermis.   Since the hyphae were growing rapidly, ramifying branches were observed easily in spite of their delicate nature.   In cells of floral bracts, hyphal strands were more numerous and generally distributed.

The fungus invaded seedling tissues causing only slight macroscopic evidence of infection.   On agar plates, mycelium developed on the surface of roots and especially coleoptiles of germinating seedlings.   Light cinnamon-brown, amorphous substances within and between cells were evident in sectioned material.   Sections of coleoptiles showed penetration of the epidermis and establishment of mycelium in the tissues (Fig. 1, F).   The fine hyphae ramified rapidly, both inter- and intracellularly through the parenchyma.   The fungus entered and ramified freely in cortical tissue around secondary roots.   Hyphae appeared in small aggregates in the cortex adjacent to the stele.   Further development in seedlings was not followed.   Seedling inoculations gave no evidence of definite necrosis or reduced vigor among seedlings of cereals or soybeans.

Seed infection was studied by inoculating florets of barley and oats.   The seed-borne nature of the fungus was established by plating and from sections of the cereal kernels.   Barley and oat flowers at anthesis were inoculated with a water suspension of ascospores.   Spore suspension was placed inside the floral bracts, without injury to the developing ovary, by means of a hypodermic needle; the inflorescences were enclosed in paper bags until the kernels were mature.   Controls using sterile water were included in the series.   Kernels from the inoculated flowers were well developed and showed no external evidence of infection.

The ripe grain was surface sterilized and plated. In one half of each lot the lemma and palea were removed completely; these structures and the naked caryopses were surface sterilized and plated on potato-dextrose agar. In the other half the hulled kernels were surface sterilized and plated. After seven days at 20° C. colonies of *Microascus* had developed from all kernels and floral bracts plated.

The fungus apparently persists within the enveloping tissue in dormant seeds of several crop plants. Blossom inoculation establishes the fungus in pericarp tissues and it is probable that natural infection occurs in a similar manner. Mycelium penetrated and ramified within the tissues of seedlings with only slight necrosis of the invaded tissues and no apparent damage to host development. This type of infection may be characteristic of a group of fungi commonly plated from seed.

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THE EFFECT OF SOAKING COTTON SEED ON THE INCIDENCE OF ANGULAR LEAF SPOT IN NEW MEXICO AND ARIZONA

A. R. LEDING AND L. A. BRINKERHOFF[1,2]

Seed disinfection by water-soaking was suggested by Boughey[3] as a possible substitute for chemical disinfectants in controlling angular leaf spot of cotton in Egypt during the recent war. Primary infection was greatly reduced in the Egyptian studies when contaminated seeds were soaked in irrigation water for 48 hours. With Boughey's experiment in mind, water-soaking was included in angular leaf spot control tests at State College, New Mexico, and at Sacaton, Arizona, during the summer of 1942. As the first year's results were not in agreement with the Egyptian experiments the tests were repeated at both stations.

MATERIALS AND METHODS

At Sacaton, Arizona, naturally infested seed of the American-Egyptian variety S × P, *Gossypium barbadense* L., was used for Test 1, and the related variety Pima for Test II. Seed of the latter was sprayed with a culture of *Xanthomonas malvacearum* (E.F.Sm.) Dowson and then thoroughly air dried. For Tests III and IV at Sacaton and Test III at State College, New Mexico, seed of S × P was similarly inoculated. Seed of the upland variety Acala 1517, *G. hirsutum* L., obtained from naturally infected plants served for the remainder of the tests at State College.

The various lots of seed were soaked at room temperature and then air dried. The 48-hour period recommended by Boughey caused sprouting at the temperatures which prevailed at Sacaton, Arizona, during April of 1942 and 1943; consequently, the soaking period was reduced to 24 hours in April and to 12 hours in May. At State College where lower spring temperatures prevail, the 48-hour period was employed. Seed for Test III at both stations came from the same lot and was soaked at Sacaton for 24 hours. A 5-minute and a 1-hour soaking period were included in Test IV at State College in 1944.

The tests were conducted in the field and the seedlings grown under ordinary farm practices, each station using methods adapted to its area. Pre-planting irrigations supplied moisture for germination and growth of the seedlings for the duration of the tests except for Test IV at State College, which was irrigated before the final count was taken.

The percentages of plants infected with angular leaf spot (Tables 1

and 2) are believed to represent primary infection in most instances. Where there remains a possibility that infection may have resulted from inoculum borne by rains or irrigation water this is indicated in footnotes accompanying each table. Three replications were used except in Tests I and II at State College which contained 2 and 5, respectively.

TABLE 1.—*The effect of soaking cotton seed on the incidence of angular leaf spot in cotton seedlings at the United States Cotton Field Station, State College, New Mexico*[a]

Test number, period, and treatment[b]	Number of seedlings	Percentage of seedlings infected with angular leaf spot
I.[c] *April 18 to May 23, 1942*		
Soaked 48 hours	2221	30.3
Not soaked	1773	4.1
II.[c] *May 1 to May 30, 1942*		
Soaked 48 hours	346	58.1
Not soaked	350	9.7
III.[d] *April 21 to May 27, 1943*		
Soaked 48 hours	1648	28.1
Not soaked	1264	1.3
IV.[e] *May 11 to June 10, 1943*		
Soaked 48 hours	1604	24.2
Not soaked	889	0.9
V.[d] *April 21 to May 26, 1943*		
Soaked 24 hours (clear water)	188	26.6
Soaked 24 hours (silty water)	199	15.6
Not soaked	177	0.6
VI.[f] *May 19 to June 19, 1944*		
Soaked 48 hours	217	18.0
Soaked 1 hour	205	10.0
Soaked 5 minutes	211	8.5
Not soaked	194	1.0

[a] The data represent a summation of 2 replicates for Test I, 5 replicates for Test II, and 3 replicates for each of the remaining tests.

[b] Naturally infested seed of the upland variety Acala 1517 was used for all except Test V which was planted with artificially inoculated seed of the American-Egyptian variety S × P.

[c] There was no rain between seedling emergence and the date the final counts were taken. All infection is considered primary.

[d] Rains occurred as follows: May 20, .02 inch; May 22, .06 inch. Probably little secondary infection occurred.

[e] Rains occurred as follows: May 20, .02 inch; May 22, .06 inch; May 29, .67 inch; May 30, .05 inch. Irrigation water was applied June 9. Secondary infection probably occurred.

[f] Rains occurred as follows: May 27, .39 inch; June 6, .40 inch. Secondary infection probably occurred.

RESULTS

Results of six trials at State College, New Mexico, are shown in table 1; table 2 presents the results obtained at Sacaton, Arizona. In all instances at State College soaking caused marked increases in the incidence of angular leaf spot, and this was also true for two of the four tests at Sacaton. The primary lesions tended to be more numerous in the seedlings from soaked seed. Angular leaf spot failed to develop in seedlings from nonsoaked seed in Test III at Sacaton in 1943, a very dry spring. The seed soaked in clear water, however, produced 16.5 per cent infected seedlings in the same test.

TABLE 2.—*The effect of soaking cotton seed on the incidence of angular leaf spot in cotton seedlings grown at the United States Field Station, Sacaton, Arizona*[a]

Test number, period, and treatment[b]	Number of seedlings	Percentage of seedlings infected with angular leaf spot
I.[c] *April 1 to May 3, 1942*		
Soaked 24 hours	39	69.2
Not soaked	45	26.7
II.[c] *April 1 to May 3, 1942*		
Soaked 24 hours	590	1.2
Not soaked	832	0.1
III.[d] *April 14 to May 11, 1943*		
Soaked 24 hours (clear water) . .	683	16.5
Soaked 24 hours (silty water)	760	6.2
Not soaked	766	0.0
IV.[d] *May 10 to June 6, 1943*		
Soaked 12 hours	1425	0.2
Not soaked	2008	0.0

[a] The data represent a summation of 3 replicates for each test, except Test I.

[b] Test I was planted with naturally infested seed while Tests II, III, and IV were planted with artificially infested seed. The American-Egyptian variety S × P was used for all but Test II which was planted with the related variety Pima.

[c] Rains occurred as follows: April 12, .10 inch; April 21, trace; April 22, .10 inch; April 23, .40 inch; April 24, .67 inch; April 29, trace. Some secondary infection probably occurred.

[d] No rain fell for the duration of the test. All infection is considered primary.

The use of silty flood water resulted in a lower percentage of the disease than did the clear water at both stations; nevertheless, there was still a significant increase over the nonsoaked seed. The two shorter soaking periods included in Test VI at State College caused increases in infection which were intermediate between the results for the check and the 48-hour soaking.

DISCUSSION AND CONCLUSIONS

Boughey[4] notes that according to a previous author (Massey, Rep. Gezira Agric. Res. Serv. 1933, 1934), the angular leaf spot organism is destroyed when cotton fields are flooded for four days, this action being attributed by that author to the action of a bacteriophage. Boughey himself, however, says, "From laboratory experiments it would appear that the organism disappears from the surface of the seed, not through the activity of a bacteriophage, but through exposure to anaerobic conditions. These conditions are the result of bacterial activity and oxygen absorption by the germinating seeds. The growth of *B. malvacearum* in culture is closely conditioned by the amount of oxygen present." Whatever the agent or mechanism effective in the Egyptian tests, it was obviously not operative under the conditions that prevailed in Arizona and New Mexico.

The fact that water-soaking cotton seed tends to increase the primary infection of angular leaf spot in Arizona and New Mexico may at times be of economic significance. Growers occasionally soak fuzzy cotton seed be-

[4] *Op. cit.*

fore planting to insure rapid germination.   The tests reported in this paper indicate that angular leaf spot may be considerably increased by this procedure.

UNITED STATES COTTON FIELD STATION,
   STATE COLLEGE, NEW MEXICO,
      AND
UNITED STATES FIELD STATION,
   SACATON, ARIZONA.

AN EVALUATION OF CERTAIN SUBSTITUTED PHENOL ESTERS FOR THE TREATMENT OF COTTON SEED[1]

C. H. ARNDT

One of the objectives in recent experimentation with fungicides has been the discovery of a chemical that would be less irritating to the operator and less toxic to animals than most of the fungicides now in general use. This applies especially to the treatment of cotton seed, for which there are frequently surpluses of unplanted treated seed that could be profitably used for oil extraction. Preliminary tests have indicated the possibility of the extraction of marketable oil from cotton seed treated with zinc 2,4,5-trichlorophenate; while laboratory tests (8) and field plantings (4) indicate that it is an effective fungicide for seed treatment. The irritating properties of commercially available dusts that contain this chemical may cause some inconvenience in their use; nevertheless, the hazards associated with their use are relatively small. This paper presents results of studies made to ascertain whether available esters of 2,4,5-trichlorophenol and related phenols might be effective fungicides and be less irritating to the operator when used for the treatment of cotton seed.

The seed of the cotton plant when infested by *Colletotrichum gossypii* South. supply an excellent criterion of the effectiveness of a fungicide, since (a) seed copiously infested by this pathogen are usually available, and the virulence of the fungus is maintained when the seed are stored at 1°–3° C. (2), (b) seedling infection by this pathogen is readily obtained under standardized conditions (1), and (c) the lint remaining on the seed after ginning makes it essential that an effective fungicide be volatile to some degree.

METHODS

Results obtained when seed infested by *Colletotrichum gossypii* were germinated on water agar (1) and unpublished results obtained when similar seed were grown in sand culture have shown that a soil temperature of 24° C. approximates the optimum for the maximal infection of seedlings by this pathogen. Accordingly, treated seed were germinated in sand cultures that were placed in cases in which air temperature was maintained at 24.5° C., while the sand temperature was generally about 0.5° C. lower, because of evaporation from the cultures.

The data for seedling emergence and infection for each chemical are based on a triplicate planting of 32 seeds. Each group of 32 seeds was grown in an aluminum container, 18 cm. in diameter and 10 cm. deep, into which was placed 2500 gm. of steamed river sand. The water holding capacity (field capacity) of the actual mass of sand, as used, was 31.3 per

cent. Water was added to obtain a moisture content of 80 per cent of this amount to assure that there would be adequate aeration for rapid germination. The water added, 625 cc., contained mineral nutrients in the following concentrations: KNO_3, 0.002 M; $NH_4H_2PO_4$, 0.001 M; $Ca(NO_3)_2$, 0.004 M; and $MgSO_4$, 0.001 M. After the water was added, the sand was thoroughly stirred with an icepick and its surface leveled. Preliminary experimentation had shown that a thorough stirring of the sand is essential for rapid and uniform seedling emergence, probably because of the increased aeration of the sand. A planting guide into which 32 regularly spaced 12-mm. holes had been drilled was placed on the surface of the sand; one seed was dropped into each hole; and the seeds were finally forced into the sand to a depth of 2.5 cm. with a wooden punch 10 mm. in diameter. The seeds were covered with sand by stirring its surface with an icepick.

A relative humidity of about 75–80 per cent was maintained in the cases and the water loss from each culture after 7-8 days amounted to 100 to 150 cc. At this time the sand was brought to the initial moisture content by sprinkling water on its surface. Preliminary tests had indicated that the addition of water at this time with the associated packing of the moist sand around the bases of the hypocotyls tended to increase the number and severity of the anthracnose lesions, which improved the evaluation of the chemicals under test.

The cultures were not illuminated until after seedling emergence was almost completed, usually the 5th day, after which the cases were irradiated by three, 40-watt fluorescent lamps 10 cm. above the glass tops of the cases, or about 70 cm. above the seedlings. Irradiation did not seem to influence the final results, but did induce a more normal type of seedling growth. Seedling counts were made after 4, 5, 6, 7, 10, and 12 days to observe any effect of treatment on seedling emergence and on the severity of infection. On the 12th day the seedlings were removed from the sand to obtain data on seedlings that had lesions on the hypocotyls. Since 12 days were sufficient for maximal seedling infection but not for maximal injury to seedlings, no additional information would have been obtained by a longer growth period. Since the earlier counts showed no definite effect for any chemical at non-toxic dosages on the rate of emergence, the data, unless otherwise specified, indicate the total number of seedlings that were infected on the 12th day. Control cultures of untreated seed were grown in each test. The number of emerged seedlings was generally about the same as that for the more effective fungicides, but the number of uninfected seedlings was small, ranging from 0 to 6 for the particular lot of fuzzy[2] Delfos 651 seed used. This seed was stored at 3° C. until treated with the chemicals.

For convenience in making comparisons among the chemicals studied, all dosages are reported in grams of the active chemical per kilogram of

[2] Seed from which the longer seed-coat hairs have been removed in ginning, but not the shorter hairs that constitute the linters.

seed, although the chemicals were diluted with the requisite amount of micronized pyrophyllite to permit the application of the dust at a rate not less than 3 gm./kg. of seed, a rate application well below the maximal that would be retained by seed. The chemicals,[3] when initially prepared, were mixed with an equal weight of micronized pyrophyllite, after which the mixture was ground in a ballmill until a suitable dust was obtained. All dusts adhered well to the seeds. Other dilutions were obtained by adding the requisite amount of prophyllite to the 50 per cent dust. After the requisite amount of dust was added to the seeds (usually 200 gm.), the seeds were agitated for 10 minutes in a mechanically rotated churn. The seeds were then stored in a paper container at 20°–26° C. for 24 hours before planting.

The comparatively sharp melting points of most of the chemicals used, table 1, would indicate that they were relatively pure. This does not apply to the 2,3,4,6-tetrachlorophenyl acetate, which yielded materials of widely different melting points on recrystallization. It is assumed that the effects reported are due to this chemical, but the possible toxicity of impurities is not precluded. Although the planting unit for a single test of a given dosage of each chemical was a triplicate planting of 32 seeds, most of the results were confirmed by comparable data from two or more tests, particularly at the 2 gm./kg. dosage.

Statistical analyses of the data showed that differences in emergence of less than 15 per cent were not significant at the 1 per cent level. This relatively high difference required for significance was largely due to the difficulty of detecting all manifestly defective seeds before planting. Since the criterion of fungicidal effectiveness in these tests was the protection afforded seedlings against infection by *Colletotrichum gossypii,* the original data were recalculated to show the percentage of infection. Analyses of these percentages showed that a difference of 5.5 per cent was significant at the 1 per cent level.

<div align="center">RESULTS</div>

A much greater effectiveness in reducing seedling infection by *Colletotrichum gossypii* for the formate, acetate, and propionate esters of 2,4,5-trichlorophenol than for the laurate, benzoate, succinate, and carbonate esters is indicated by the data on seedling infection, table 1. The results for the benzoate may not be entirely comparable to those for the other esters, since the dust prepared from this chemical was somewhat less dusty than that prepared from the other chemicals.

The position and number of the chlorine substituents influenced the fungicidal activity to a lesser degree than did the acidic radical. Thus, the 2,4,6-tri- and 2,3,4,6-tetrachlorophenyl acetates were almost as effective as the 2,4,5-ester; while the 2,3,6-tri- and pentachlorophenol esters were slightly less effective. Similarly, the substitution of bromine for chlorine

in the 2,4,5- and 2,4,6-trichlorophenyl acetates did not greatly affect the fungicidal activity.

Seeds treated with Ceresan-M (7.7 per cent ethyl mercury p-toluene sulfonanilide) and Dow 9B (50 per cent zinc 2,4,5-trichlorophenate) at a rate of 3 gm./kg. were also included in several of the tests. The emergence for seed treated with either of the dusts averaged about 75 per cent, and 2 to 4 per cent of the seedlings were infected by *Colletotrichum gossypii*. This percentage of infection is without significance, since a comparable test with acid-delinted seed indicated that a small percentage of

TABLE 1.—*Results from the germination in steamed sand of 96 cotton seeds infested by Colletotrichum gossypii after treatment with various phenyl esters. Dosages given indicate grams of active chemical per kilogram of seed*

Chemical	Melting point °C.	Seedlings[b]		Dosages	
		Emerged	Infected	Tested	Toxic[c]
1. 2,4,5-trichlorophenyl formate	72– 73	69	3	2–4	4
2. Do Do acetate	67– 68	75	1	0.5–4	3
3. Do Do propionate	59– 63	73	8	2–4	2
4. Do Do laurate	60– 61	74	44	2–3	. ..
5. Do Do benzoate	85– 88	72	21	0.75–3.0	3+
6. Bis(2,4,5-trichlorophenyl) carbonate	170–171	57	36	2–8	8+
7. Bis(2,4,5-trichlorophenyl) succinate	138–139	53	47	2–3	. ...
8. 2,4,5-tribromophenyl acetate	98–100	71	2	0.32–3	3
9. 2,3,6-trichlorophenyl acetate	40– 42	72	11	2	... .
10. 2,3,6-trichlorophenyl benzoate	89– 91	69	51	2	... ...
11. 2,4,6-trichlorophenyl acetate	49– 52	65	2	0.5–4	2
12. 2,4,6-trichlorophenyl benzoate	71– 74	73	46	2	..
13. 2,4,6-tribromophenyl acetate	82– 85	73	12	2–4	4
14. 2,3,4,6-tetrachlorophenyl acetate	70–110[a]	76	3	0.25–2	0.72–1
15. Pentachlorophenyl acetate	148–151	59	9	2–4	2
16. o-nitrophenyl acetate	37– 39	73	43	1–2	2
17. p-nitrophenyl acetate	77– 80	71	16	0.5–2	2
18. 2,4-dinitrophenyl acetate	71– 73	72	20	2	2
19. 4-chloro-3,5-dimethylphenyl acetate	47– 48	73	16	2	... ...

[a] Prepared from technical grade chemical, Dowicide No. 6. Recrystallization of this chemical did not result in a product with a definite melting point.

[b] Dosage 2 gm./kg. of seed; except 1 gm./kg. dosages for Nos. 8, 16, and 17; and 0.5 gm./kg. for No. 14, which chemicals were toxic at the higher dosages.

[c] Toxic to cotton seedling as indicated by stunting or discoloration of hypocotyl.

the seeds were internally infected by this fungus; and no fungicide thus far tested has prevented the growth of this fungus on seedlings that develop from internally infected seeds.

The dosages at which the chemicals were toxic to the seedlings varied from 0.75 gm./kg. for the tetrachlorophenyl acetate to 2 gm./kg. or higher for most of the other chemicals. The carbonate ester showed no toxicity at 8 gm./kg. The first indication of toxicity was a slightly delayed emergence, which was usually associated with abnormal directional growth of the primary root. The direction of the growth might be horizontal or even almost vertical for several centimeters (Fig. 1). At dosages that were only slightly toxic, this retardation of growth was only temporary, and the seedlings

grew as rapidly as those at non-toxic dosages after normal directional root growth was assumed. Six chemicals, 2,4,5-trichlorophenyl acetate, 2,4,5-tribromophenyl acetate, 2,4,5-trichlorophenyl propionate, tetrachlorophenyl acetate, pentachlorophenyl acetate and o-nitrophenyl acetate, tended to cause a discoloration of the hypocotyl in proximity to the treated seed coat at dosages which approximated those at which abnormal root growth occurred. These blackish discolorations, at times, extended upward in lines on the hypocotyls for several centimeters above the position of the seed coat. The 2,4,6-trichlorophenyl acetate caused no discoloration at toxic dosages, although the seedlings were definitely stunted and the hypocotyls of smaller diameter than those of normal seedlings.
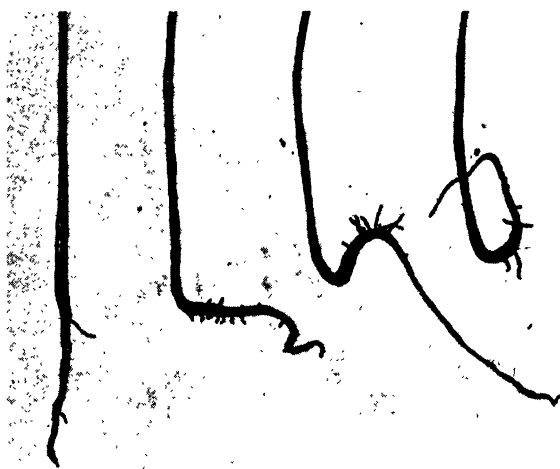
FIG. 1. Abnormal directional root growth of seedlings that developed from seed treated with 2,4,5-trichlorophenol at 3 gm./kg. Normal root at left.

After the initial tests had indicated the fungicidal properties of the four chemicals listed in table 2, further studies were made to ascertain the range of effective dosages and the maximal dosage that would not be toxic to the cotton seedling. The results show almost no safety factor for the 2,4,6-trichlorophenyl acetate, since it was not fully effective at 1.5 gm./kg. and was toxic at 2.0 gm./kg. The tetrachlorophenyl acetate with an effective dosage of about 0.5 gm./kg., with an indication of toxicity at 0.75 gm./kg., and with a definite toxicity at 1.0 gm./kg., had only a slightly greater safety factor. In contrast, the 2,4,5-trichlorophenyl acetate had a safety factor of at least 3, since it was effective in all tests at 0.75 gm./kg. and had no definite toxicity to the seedlings until a dosage of 2.5 to 3.0 gm./kg. was used. This ester varied greatly in its effectiveness at 0.5 gm./kg; the infected seedlings in several tests ranged from 5 to 31 per cent, a variation that might be expected at a dosage approximating the minimal effective dosage. The 2,4,5-tribromophenyl acetate had a somewhat greater safety factor than the corresponding chlorine compound, since it was more

effective than the latter compound at dosages below 0.75 gm./kg; and it became toxic at the same dosage, or 3 gm./kg.

In another test, dosages of 2,4,5-tri- and 2,3,4,6-tetrachlorophenyl acetates at slightly less than the effective dosages, or 0.5 gm. and 0.37 gm., respectively, were applied simultaneously in the same dust to 1 kg. of seed. Only 3 per cent of the hypocotyls of these seedlings were infected which seems to suggest some degree of synergism. The application of 1.5 gm. and 0.75 gm., respectively, in the same dust resulted in the prevention of all seedling infection, but 60 per cent of the roots grew abnormally and 40 per cent of hypocotyls had blackened bases, which would indicate that their toxicity to the host plant is also additive.

The seedlings in the cultures planted to seed treated with the carbonate ester appeared to be injured earlier and more severely by the anthracnose fungus than in those planted to nontreated seed. In order to study this

TABLE 2.—*Comparative percentages of seedlings infected by Colletotrichum gossypii when the seed were treated with the several dosages of the chemicals indicated*[a]

Chemical	Dosage in gm./kg.						
	0.37	0.50	0.75	1.00	1.50	2.00	3.00
2,4,5-trichlorophenyl acetate		15	3	3	4	1	0[b]
2,4,6-trichlorophenyl acetate		57	.	13	8	2[b]	.
2,3,4,6-tetrachlorophenyl acetate .	10	3	3[b]	3[b]	.	.	
2,4,5-tribromophenyl acetate	8	7	3	2	4	3	0[b]

[a] The percentages in most instances are means for 3 to 4 tests. For convenience in summarizing the data, in several instances results at a lower dosage were combined with those at the indicated dosage, when the difference in dosage did not exceed 10 per cent.

[b] Slightly toxic to the cotton seedling at this dosage.

more fully, seeds were treated with the dosages indicated in table 3. The results seem to indicate a predisposing effect of the carbonate ester, since after 8 days more seedlings from the seeds treated with the carbonate had visible lesions on the base of the hypocotyl than did the seedlings from nontreated seed. The 8-gm. dosage did not retard emergence.

During these tests a number of other related compounds were tested at a dosage of 2 gm./kg. under similar conditions. These chemicals and the percentages of infected seedlings were as follows: 2,4,5-trichlorophenyl methyl ether, 61; pentachloronitro benzene, 51; bis(5-chloro-2-hydroxyphenyl) methane, 13; bis(5-chloro-2-acetoxyphenyl) methane, 64; and bis(5-chloro-3,5,6-trichlorophenyl) methane, 18. Thus, only the third and fourth chemicals of this series showed any effectiveness, which was not adequate for the treatment of cotton seed, regardless of their use for the mildew-proofing of fabrics (7) and in germicidal soaps (5). The seedlings that developed from the seeds treated with the latter of these two chemicals had chlorotic spots as large as 0.5 cm. in diameter on their cotyledons. This was the only specific pathological effect noted on the tops of the seedlings for any of the 24 chemicals included in these tests.

In order to ascertain whether the results obtained with Seedox[4] in the laboratory might be used to evaluate its effectiveness in field plantings, two lots of Coker 100-WR cotton seed naturally infested by *Colletotrichum gossypii* were used in the field plantings in 1947. Part of the nontreated fuzzy seed of each lot was run through a close-set gin to remove most of the longer seed-coat hairs and obtain the reginned seed. Portions of these two kinds of seeds were treated with the required amount of Seedox to apply the active chemical at rates of 0.5, 1.0, and 1.5 gms. per kg. of seed; other portions were treated with Ceresan-M and Dow-9B, each at dosages of 3 gm./kg., and were included in the plantings for comparisons with Seedox. The treated sub-lots of seed, thus obtained, were germinated in sand culture as described for the previous tests.

The seedlings obtained in the laboratory from the nontreated fuzzy and

TABLE 3.—*Effect of bis(2,4,5-trichlorophenyl) carbonate on the infection of cotton seedlings by Colletotrichum gossypii. Data are based on 96 seeds*

Dosage gm./kg.	Seedling emergence		Seedlings infected	
	Days 6	Days 8	Days 8	Days 10
	No.	*No.*	*No.*	*No.*
0	63	67	27	60
2	70	70	39	68
4	62	62	38	62
8	73	73	44	73

reginned seed of lot A were mostly infected by the anthracnose fungus (Table 4); for lot B a much smaller percentage of the seedlings from the reginned seed were infected than of those from the fuzzy seed. Such differences in the effect of reginning on the relative number of seedlings infected for fuzzy and reginned seed are not unusual. Only small percentages of seedlings were infected in the cultures planted with seed treated with Ceresan-M, Dow-9B, and the two higher dosages of Seedox. The somewhat higher percentages of seedlings that were infected for the fuzzy and reginned seed of lot B treated with the lowest dosage of Seedox indicate that this dosage is too low for effective seed treatment.

In these plantings, there were only three instances in which the percentage of seedlings for a treatment was significantly greater than the percentage for another; namely, Ceresan-M and 2 × Seedox relative to Dow-9B on the fuzzy seed of lot A in the April 25 planting and Ceresan-M relative to 1 × Seedox on the reginned seed of lot B in the April 18 planting. Thus, the number of significant differences among these treatments are too few to indicate any constant superiority for any of them. The relatively low number of seedlings for the 1 × dosage of Seedox on the fuzzy and reginned seed of lot B in the April 18 plantings adds support to the laboratory

[4] Trade name of dust containing 50 per cent of 2,4,5-trichlorophenyl acetate.

results which indicated that this dosage was not great enough to completely destroy the anthracnose fungus on the seed coat.

In contrast to the few significant differences among treatments in the percentage of surviving seedlings, the percentages for the nontreated seed were significantly below those for the best treatment of the same kind of seed in all plantings. The percentages of surviving seedlings for the nontreated reginned seed of lot B were less than the percentage of noninfected seedlings (30 per cent infected) in the laboratory for the same sub-lot of

TABLE 4.—*Comparative results for Seedox, Dow 9B, and Ceresan-M when used to treat portions of two lots of cotton seed*

Type of seed and treatment	Seedlings in sand culture in laboratory at 24°C.				Seedlings surviving in the field (percentage of seeds planted[b])				
	Percentage emerged[a]		Percentage infected by *C. gossypii*		Planting date				
					Apr. 18		Apr. 25		Apr. 26
					Lots				
	A	B	A	B	A	B	A	B	B
Fuzzy seed									
a. Check	65	80	54	79	40	28	39	50	42
b. Ceresan-M	77	92	0	4	56	61	62	68	71
c. Dow 9B	71	84	0	4	59	64	44	63	72
d. Seedox-1 ×[c]		85		17		55		58	72
e. Seedox-2 ×	74	91	3	1	64	67	59	67	75
f. Seedox-3 ×		84		3		70		60	72
Reginned seed									
a. Check	84	80	77	30	37	31	27	39	47
b. Ceresan-M	80	83	1	0	57	60	46	58	68
c. Dow 9B	74	81	0	6	56	47	38	54	68
d. Seedox-1 ×		77		9		43		46	61
e. Seedox-2 ×	73	78	1	0	45	55	38	50	60
f. Seedox-3 ×		73		0		48		43	67
L.S.D.[d]					13	17	16	12	15

[a] Based on the germination of 32 seeds in triplicate.
[b] Based on a quadruplicate planting of approximately 500 seeds.
[c] 1 ×, 2 ×, and 3 × indicate dosages of 0.5, 1.0, and 1.5 gm./kg. of seed, respectively, of 2,4,5-trichlorophenyl acetate, which was applied in a dust containing 50 per cent pyrophyllite as a diluent.
[d] Amount required for significance at 1 per cent level between any two treatments.

seed; while the percentages of surviving seedlings for the other three nontreated sub-lots exceeded in all plantings the percentages of noninfected seedlings in the laboratory. Since these sub-lots were germinated under approximately optimal conditions for seedling infection by the anthracnose fungus, it may be assumed that the seedlings were less severely injured by this fungus in the field than in the laboratory tests, since infected seedlings did not survive in the latter tests. Similar differences between nontreated and treated seed have been obtained in comparisons between field plantings and laboratory tests of previous years. Since these data all indicate a tend-

ency for the seedling infection to be more severe in this particular laboratory technique than in field plantings, this technique may be considered a more rigorous test of the effectiveness of a fungicide in preventing seedling infection by seed-borne mycelia of *Colletotrichum gossypii* than are most field plantings; but it does not necessarily indicate the protection that a given fungicide may afford seedlings against infection by soil-inhabiting pathogens.

<div align="center">DISCUSSION</div>

The effective dosage of 2,4,5-trichlorophenyl acetate for the treatment of cotton seed is almost identical with the dosage of 0.6–1.5 gm. per kilogram of fuzzy seed that Meuli and others (8) found best for the zinc salt of the same trichlorophenol. Their data for the latter salt would indicate a somewhat greater tolerance of the cotton seedling for this chemical than for the acetate (8), since their data show no depression of germination until a dosage of 9 gm./kg. was applied in a 75 per cent dust. In their test, it is questionable, however, whether all of the dust adhered to the seed at dosages above 6 gm./kg. In a field planting of fuzzy cotton seed that had been treated with Zn 2,4,5-trichlorophenate at rates of 1.5, 3.0, and 6.0 gm. per kilogram of seed, the percentages of emerged seedlings were 65, 55, and 37, respectively; which indicates that the zinc salt of 2,4,5-trichlorophenol has about the same toxicity to the cotton seedling as the acetate. Meuli et al. (8) found that the Na salt of 2,4,6-trichlorophenol was somewhat less effective on cotton seed at dosages of 1.0 gm./kg. than the Na salts of the 2,4,5, the tetra-, and pentachlorophenols, which corresponds with their relative effectiveness as esters. In contrast to the results of these tests, which showed a much greater toxicity of the tetrachlorophenol than of pentachlorophenol ester to cotton seedlings, Meuli et al. report greater toxicity to pea seedlings for the pentachlorophenol.

These chlorophenols also have been tested for the control of wood-rotting fungi. Baechler and Bateman (3) found that 0.002 per cent tetra- or pentachlorophenols in nutrient agar inhibited the growth of the fungus studied and that these were among the cheapest of the effective fungicides. These studies were extended by Hatfield (6) to include other chlorophenols. His data show a comparable toxicity for the 2,4,5-tri- and the tetrachlorophenols and a lesser toxicity for the 2,4,6-trichlorophenol. The concentration of pentachlorophenol necessary to inhibit the test fungi was greater than for the other phenols, but the greater insolubility of this chemical made comparisons uncertain. The concentration of the more effective chemicals, the Na salts of 2,4,5-tri- and the tetrachlorophenols, necessary to inhibit the fungi on the agar medium was 0.001–0.002 per cent, or much less than the effective dosages on cotton seed for the 2,4,5-tri- and tetrachlorophenyl acetates, or 0.075 and 0.05 per cent, respectively.

There is no evident correlation between the melting points of the chemicals and their fungicidal activity (Table 1). The 2,4,5-trichlorophenyl acetate has a slightly higher melting point than the 2,3,6- or the 2,4,6-

trichlorophenyl acetate; yet it is the most effective of these acetates. There is, however, a very definite correlation with the position of the chlorine substitutents in the benzene ring. The acid entering into the formation of the ester is also important; the simpler acids, such as formic and acetic, form more effective fungicides than those of higher molecular weight, such as lauric and benzoic acids. The data, however, give no indication of the physical-chemical properties of the phenol derivatives on which their fungicidal activity might be predicated. No irritation was observed while treating the seed with any of the esters that had been prepared from relatively pure chemicals, except for the noticeably pungent formate.

SUMMARY

Cotton seeds naturally infested by *Colletotrichum gossypii* were treated with various substituted phenyl esters and the seeds germinated in sand culture at 24° C. At comparable dosages, the acetate of 2,4,5-trichlorophenol was effective in preventing seedling infection by *C. gossypii*; the formate and propionate esters were slightly less effective; while the carbonate, succinate, and laurate were not effective. The substitution of bromine for chlorine caused relatively little change in effectiveness. The position of the Cl atoms in the benzene ring also influenced fungicidal properties. The 2,3,4,6-tetrachlorophenyl acetate was more toxic to both the fungus and the host plant than the 2,4,5-trichlorophenyl acetate; while the chemicals with the Cl atoms in the 2,4,6, the 2,3,6 and the 2,3,4,5,6 positions were somewhat less effective fungicides. Effective fungicides were not obtained when NO_2 was substituted for Cl in chlorophenyl acetate. Results obtained in field plantings were generally in agreement with the laboratory results and indicated that 2,4,5-trichlorophenyl acetate at a dosage of 1 gm./kg. of seed is a satisfactory treatment for the elimination of the infestation of fuzzy cotton seed by *C. gossypii*.

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STRAINS OF MYCOSPHAERELLA FRAGARIAE[1]

A. G. Plakidas

In the course of many years' work with diseases of strawberries, the writer obtained observational evidence suggestive of the existence of strains of the leaf spot fungus, *Mycosphaerella fragariae* (Tul.) Lindau, varying in their pathogenicity on different varieties of strawberry. Other workers, also, had apparently made similar observations. In private correspondence with the writer, G. M. Darrow pointed out that certain varieties and seedling selections might have a high degree of resistance to this disease in the field in Mississippi and extreme susceptibility in North Carolina, or Maryland, or vice versa. This was suggestive, at least, that different pathogenic strains of the fungus existed in different regions of the country.

Information on whether or not the leaf spot fungus consists of strains differing in their pathogenicity to different varieties of strawberry is of great practical significance. Strawberry breeding projects are in progress in many State experiment stations as well as in the United States Department of Agriculture. Because of the destructiveness of the leaf spot disease in many of the strawberry growing areas of the country, one of the chief objectives of the breeders is to produce varieties that are resistant to this disease. It is, therefore, pertinent to know whether one is dealing with a pathogen which is constant in its reaction or with one composed of strains of varying pathogenicity in respect to different varieties.

It was the object of this study to obtain experimental evidence of whether or not strains of *Mycosphaerella fragariae* exist. It is believed that the data obtained show rather conclusively that this fungus is composed of strains that differ in their pathogenic reaction to different varieties of strawberry.

MATERIALS AND METHODS

Fungus Strains. The following seven strains (isolates) of the leaf spot fungus were used:

S3—isolated from typical leaf spot lesions on Klondike variety of strawberry, from Hammond, Louisiana, 1935.

S18—isolated from atypical lesion (large, brown spots without the grayish center and purple margin) on Klondike, from Hammond, Louisiana, 1935.

S83—isolated from typical lesions on leaves of a seedling strawberry (No. 820) from Corvallis, Oregon, 1938.

S1388—single-ascospore isolate from dead leaves of wild strawberry (*Fragaria virginiana*) from East Lansing, Michigan, 1939.

S1502—ascospore isolate, culture obtained from Dr. J. B. Demaree, Beltsville, Maryland, 1939.

S1600—isolated from leaves of Geneva Station seedling No. 19747, from Geneva, New York, 1940.

S1610—isolated from leaves of Klondike, from Hammond, Louisiana, 1940.

These seven isolates appeared alike morphologically when grown on the same medium and under the same conditions. All produced *Ramularia* conidia in great profusion. However, no detailed statistical comparison of size and number of conidia or of other characters was made.

Strawberry Varieties. The following ten varieties were used as differential hosts: Klondike, Klonmore, Howard 17 (Premier), Marshall, Dresden, Caledonia, Clermont, U.S.D.A. seedlings Nos. 1021 and 1942, and New Jersey seedling No. 303.

The reactions of the first four varieties to the fungus, under Louisiana conditions, were known from previous experience. The other six were selected in the variety test field of the Pomology department at Geneva. Leaf spot was rather prevalent in this field and it was assumed that all the varieties in it were equally exposed to infection. Therefore, in selecting varieties to be used in the inoculation experiments, those that were free of disease were tentatively considered resistant and those that were badly spotted were considered susceptible. Accordingly, the ten varieties were tentatively grouped, at the beginning of this study, as resistant or susceptible as follows: Klonmore, Howard 17, Dresden, and U.S.D.A. seedlings Nos. 1021 and 1942 were considered resistant; Klondike, Caledonia, Clermont, Marshall, and New Jersey seedling No. 303 were considered susceptible.

Examination of the results of the inoculation experiments (Table 1) will show that with few exceptions these varieties failed to maintain their position in this tentative grouping in response to the different strains of the pathogen. Thus, the choice of these varieties as differential hosts proved a happy one.

Procedure. The fungus was grown on hard (4 per cent) agar in Petri dishes. Beanpod and potato-dextrose agars were used. About 0.5 cc. of a conidial suspension in sterile water was placed in each dish and smeared on the surface of the agar by rotating the plate. In about a week, the surface of the agar was covered with growth which produced conidia in great profusion. When ready to use, sterile water was poured into the dish and, by means of a soft brush, the conidia were brought into suspension.

The inoculum was applied to the plants by means of a glass atomizer attached to a compressed air line equipped with a reducing valve to regu-

late air pressure. Both sides of the leaves were covered with inoculum. The plants were growing well in 7-inch pots on rich garden soil. Two plants of each variety for each strain of the fungus were used in each experiment, and the experiments were repeated at approximately monthly intervals four to six times. Immediately after inoculation, the plants were placed in a large moist chamber of saturated humidity and kept there for 3 days. The temperature of the chamber could be regulated within certain limits, and in these experiments, it was maintained between 75° F. and 80° F. In experiments during the winter months (February–April) at Cornell, the plants were kept in the greenhouse, and daylight was supplemented by 4 hours of artificial illumination. In May and June at Cornell, and in the experiments in Baton Rouge, Louisiana, during the fall and winter, the plants were kept outdoors.

TABLE 1.—*Summary of the results of inoculation experiments with 7 isolates of Mycosphaerella fragariae on 10 varieties of strawberry as differential hosts*

Variety	Isolate						
	S3	S18	S83	S1388	S1502	S1600	S1610
Klondike	++	++	+	+	+	++	++
Klonmore	++	++	T	+	+	+++	+++
Dresden	++	+	T	+	+	+++	++
Caledonia	++	++	+++	+++	+++	+++	++
Clermont	0	0	0	T	+++	+++	0
Howard 17	0	0	0	0	T	T	0
Marshall	++	++	+	+++	T	+++	++
U.S.D.A. Seedling No. 1021	0	0	0	0	0	T	0
U.S.D.A. Seedling No. 1942	+	+	0	0·	+	+++	+++
New Jersey Seedling No. 303	+	++	T	++	+++	+++	++

Where infection occurred, lesions began to appear usually between the 12th and 15th day after inoculation, and final records were taken 28 days after inoculation. A rather coarse system of recording degree of infection was used, viz: O = No infection; T = trace of infection, not more than 5 lesions on entire plant; + = light infection, estimated less than 100 lesions per plant; ++ = moderate infection, estimated more than 100 lesions per plant but less than in next class; +++ = heavy infection, estimated several hundred lesions per plant.

The crudeness of this system is acknowledged, but, since all the readings were made by the same individual, it is considered adequate.

DISCUSSION OF RESULTS

It is believed that the results of the inoculation experiments, summarized in table 1, indicate rather conclusively that *Mycosphaerella fragariae* is composed of strains that vary in their pathogenicity toward different varieties of the cultivated strawberry. The reaction of the variety Cler-

mont is particularly clearcut and striking. This variety was heavily spotted from natural infection in the variety test field at Geneva, and it was, therefore, selected as a susceptible host to be used in this study. In the inoculation tests, however, Clermont proved resistant to 5 of the 7 strains of the fungus, but very susceptible to 2, the Beltsville and the Geneva strains. A similar reaction was exhibited by U.S.D.A. seedling No. 1942. It proved resistant, or weakly susceptible, to 5 of the 7 strains, including the Beltsville (S1502) strain, to which Clermont was very susceptible; but it was very susceptible to the Geneva (S1600) strain and one Louisiana strain (S1610).

The Oregon strain (S83) was the least pathogenic, of all the strains used, toward all varieties except Caledonia on which it was very pathogenic.

The Klonmore variety is a cross between Klondike and Blakemore and probably inherited its resistance to leaf spot from the latter, which, in turn, has the highly resistant Howard 17 as one of its parents. In the field in Louisiana, Klonmore generally remains free from leaf spot. Occasionally, it has a limited number of spots, but these usually remain of small size and solitary, and very few conidia are produced on them. However, when inoculated artificially under high humidity conditions, Klonmore is extremely susceptible; the lesions produced are large and typical of the disease, and the pathogen sporulates abundantly on them.

It is not known what factor (or factors) is responsible for the field type of resistance possessed by Klonmore and some other varieties. The results of inoculation certainly do not indicate a chemical type of resistance. Observational evidence indicates that the growth habit of the plant may account for the field type of resistance. Klonmore has an upright type of growth which aids in the quick drying of the leaf surfaces; and it has been shown[2] that the strawberry leaf spot pathogen may cause infection but apparently remain dormant in the tissue without producing external-symptoms (lesions) under conditions of low atmospheric humidity. Regardless of its nature, field resistance is of great economic significance. The case of Klonmore is an excellent example of the fact that it is unwise for strawberry breeders to depend on artificial inoculation as a means of eliminating hybrids susceptible to this particular disease. The Klonmore has become the leading commercial variety in Louisiana, occupying at present about 80 per cent of the total strawberry acreage. If artificial inoculation had been used as the criterion, this valuable variety would certainly have been lost.

The reaction of the variety Dresden is probably analogous to that of Klonmore. This variety was completely free from leaf spot in the variety test field at Geneva, and it was, therefore, selected as a resistant host. In the inoculation tests, however, it proved susceptible to all strains of the

[2] Plakidas, A. G. The mode of infection of *Diplocarpon earliana* and *Mycosphaerella fragariae*. Phytopath. 24: 620–634. 1934.

pathogen including the Geneva strain (S1600) which was present in this field.

Even more difficult to understand is the type of resistance in Howard 17 and U.S.D.A. seedling No. 1021. These two varieties have a degree of resistance approaching immunity both in the field and when inoculated with every one of the strains used in these experiments. This would suggest a chemical type of resistance. However, in a few instances in which a limited number of lesions developed from the inoculation (Table 1), the spots were typical of the disease in size and color, and the pathogen sporulated normally on them. No attempt is made to explain this phenomenon.

Without attempting to explain the nature of resistance, the strawberry varieties used in these experiments may be grouped in three general classes.

I. Those that have resistance both to natural infection under field conditions and to infection by artificial inoculation. Howard 17 and U.S.D.A. Seedling No. 1021 are examples of this class.

II. Those that have a high degree of field resistance but are very susceptible when artificially inoculated. The Klonmore, and probably Dresden, exemplifies this type of resistance.

III. Those that are susceptible both to natural infection in the field and to infection by artificial inoculation. Klondike, Caledonia, and Marshall are good examples of this class.

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THE MOVEMENT OF CROWN–GALL BACTERIA IN ISOLATED STEM FRAGMENTS OF SUNFLOWER[1]

R . S . DE ROPP

INTRODUCTION

Studies of the movement of crown-gall bacteria in the stems of intact plants were undertaken by Smith, Brown, and McCulloch (5), who concluded that the bacteria were located within the cells of the host plant and were passively transported in tumor strands. Secondary tumors were thought to result from the growth of such strands through the tissues of the stem to sites remote from the primary tumor. Later studies by Riker (2) indicated that crown-gall bacteria first establish themselves in the stem in those water-logged areas which result from wounding. He found that the organisms were capable of moving in the tracheae for distances as great as 9 cm. when cut tomato stems were immersed in suspensions of bacteria. Robinson and Walkden (3) found that, in *Chrysanthemum frutescens* and *Nicotiana affinis,* zoogleal strands of the bacteria could be demonstrated intruding into intercellular spaces and protoxylem vessels, a finding which was later confirmed by Hill (1). Suit and Eardley (6) showed that crown-gall bacteria were capable of moving in the xylem vessels of various plants and that they could remain in the internodes of tomato plants for as long as ten weeks without producing symptoms of crown-gall.

The studies to be described in this paper were undertaken to throw light on the rate of movement of crown-gall bacteria in isolated stem fragments of sunflower cultured *in vitro.* Stem tissue was obtained from four-week-old plants of *Helianthus annuus* L. var. Russian giant. The method of preparation of these fragments and the medium on which they were grown have been described in an earlier paper (4). *Phytomonas tumefaciens,* strain Brown Peach, was employed in all these experiments. The strain was fully virulent, capable of producing tumors both on isolated sunflower stem fragments and on intact plants. Suspensions of the organism were standardized by opacity and viable counts estimated by the colony count technique.

MOVEMENT OF BACTERIA IN FRESHLY ISOLATED STEM FRAGMENTS

Fragments of sunflower stem having an average length of 9 mm. were placed upright on slopes of nutrient agar, only the physiological base being in contact with the medium. A dilute suspension of bacteria was prepared from a 48-hour culture of *Phytomonas tumefaciens* and a small loop-

ful of this suspension was placed on the upper cut surface of the isolated stem fragment. Great care was taken to ensure that the drop of bacterial suspension made contact only with the cut surface of the fragment. Colony counts indicated that the number of viable bacteria deposited on the stem by this means ranged from 10 to 100. At different intervals after the application of the suspension, the stem fragments were carefully lifted from the agar and removed. The slopes were then incubated for a week at 25° C. In those instances where the bacteria had passed through the stem fragment, colonies of *P. tumefaciens* developed on the agar at the point on which the base of the fragment formerly rested. The frequency of development of these colonies is shown in table 1.

TABLE 1.—*The movement of Phytomonas tumefaciens through 9-mm. stem fragments of sunflower cultured in vitro*

Interval between application of bacteria and removal of stem fragment from agar	Number of tubes with colonies of *P. tumefaciens*	Number of tubes without colonies of *P. tumefaciens*
None	0	9
4 min.	8	1
30 min.	6	4
1 hr.	3	6
4 hr.	9	1
6 hr.	2	8
8 hr.	6	3
12 hr.	5	5
24 hr.	11	5

The results indicate that crown-gall bacteria were capable of passing through a 9-mm. fragment of sunflower stem in as little as four minutes after being applied to the upper surface of the fragment. Penetration did not occur regularly and there were instances where the bacteria failed to pass through the stem even in 24 hours. This irregularity was probably due to the small number of organisms used. Sections of the material made from 24 to 48 hours after application of the bacteria did not reveal evidence of zoogleal strands within the tissues. It appears from this and from the rapidity of the penetration that these organisms passed through the stem in the tracheae as Riker (2) and Suit and Eardley (6) have suggested. In these isolated stem fragments the transpiration stream mentioned by Suit and Eardley as being largely concerned with the transport of the organisms cannot have played any part. It seems more likely that the mobility of the organisms themselves was responsible for transfer through the stem. According to Riker (2), these organisms are capable of moving at the rate of 1 mm. per minute in distilled water.

A repetition of these experiments using stem fragments placed on the agar on their physiological tops and treated with bacteria on their physiological bases gave similar results to those previously recorded. Evidently, the polarity of the stem fragments did not affect the rate of movement of crown-gall bacteria within them.

THE EFFECT OF CALLUS FORMATION ON PENETRATION OF CROWN-GALL BACTERIA

To determine whether the formation of callus on the cut surface of isolated stem fragments would prevent the penetration of crown-gall organisms, an experiment was set up in which the bacteria were applied to the upper surface of the fragment after it had been cultured *in vitro* for various periods. These stem fragments were not removed from the agar after having been treated with bacteria, and in those instances where the bacteria passed through the fragment, mucilaginous masses of the organisms appeared growing at its base. Suspensions of bacteria were prepared as described in the previous section and the number applied to the upper surface of the stem fragment ranged from 10 to 100 viable organisms. The drop of bacterial suspension was applied in such a way that the metal of the loop did not touch the surface of the stem tissue. Bacteria were applied to the stem fragments immediately after they had been set up and after they had been cultured for three days, one week, and two weeks. The extent to which the bacteria penetrated the stem fragments under these conditions is shown in table 2.

TABLE 2.—*Effect of preliminary culture of isolated stem tips on the penetration of Phytomonas tumefaciens*

Period of culture of stem fragments prior to application of bacteria	Number of stem fragments penetrated by bacteria	Number of stem fragments not penetrated by bacteria
0 days	20	0
3 days	7	12
1 week	0	18
2 weeks	1	18

Evidently after about three days the callus pad which forms on the cut surface of the isolated stem fragment began to present a barrier to the penetration of the crown-gall organism. After a week, this barrier became almost impenetrable. Histological studies revealed that callus formation began to block the open vessels about three days after stem segments were placed on agar. It was presumably this blockage of the vessels that prevented the penetration of the bacteria.

To determine whether the callus that formed at the base of the isolated stem fragment would also act as a barrier to bacterial penetration, this experiment was repeated using stem fragments from which the upper callus pad was removed just prior to the application of the bacteria. The number of stem fragments penetrated under these circumstances is shown in table 3.

Apparently the lower callus pad formed a barrier which was just as effective in preventing the penetration of bacteria as was the upper pad. Bacteria entered the stem fragment and could be isolated from it. They also gave rise to tumors at the cut surface. But owing, presumably, to

the blockage of the vascular elements, the bacteria were unable to escape from the stem fragments onto the agar.

MOVEMENT OF BACTERIA APPLIED TO THE SIDE OF THE STEM FRAGMENT

In this experiment, stem fragments 9 mm. in length were placed on their sides on agar slopes. A small drop of a suspension of crown-gall organisms containing 10–100 bacteria was placed on the upper side of these fragments at an equal distance from both ends immediately after they had been placed on the agar. Great care was taken to avoid contaminating the agar with organisms. Out of a group of 20 stem fragments treated in this way, bacteria appeared on the agar in four instances. Tumors containing crown-gall organisms developed in 11 of the remaining 17 fragments. These tumors were not located at the point of application of the bacteria but grew from the cut surfaces at the ends of the fragments. The structure and point of origin of these tumors will be described elsewhere.

TABLE 3.—*Effect of removal of upper callus pad from stem fragments on the penetration of Phytomonas tumefaciens*

Period of culture of stem fragments	Number of stem fragments penetrated	Number of stem fragments not penetrated
0 days	20	0
3 days	3	14
1 week	0	18
2 weeks	0	20

Here it is relevant to note that crown-gall bacteria applied to the lateral surface of a stem fragment from which the epidermis had been removed were capable of passing along the fragment to its ends and of initiating tumefaction at these points. The route followed by the organisms remains in doubt.

MOVEMENT OF BACTERIA IN INTACT SUNFLOWER STEMS

For comparative purposes, some experiments were carried out on the movement of bacteria in the intact sunflower stem. In one group of five plants, the bacteria were applied to the top of the first internode which had first been decapitated. The organisms were suspended in water and applied with a loop. A dense suspension was used containing in the order of a billion organisms. In a second group of five plants, the organisms were applied at the base of the internode which was cut half way through with a razor, the inoculum being introduced into the cut. These stems were removed one and twenty-four hours after inoculation. After removal of the epidermis, the stems were cut into 5-mm. fragments and each of these fragments was ground up and placed on nutrient agar. The number of fragments from which *Phytomonas tumefaciens* was recovered and the distance of these fragments from the point of inoculation is shown in table 4.

The organisms were evidently capable of moving from 5 to 6 cm. within

the internode in one hour. Their rate of movement and the distance they migrated was approximately equal whether they were applied at the base of the internode or the top. The distance of migration and rate of movement of these organisms in sunflower agree fairly closely with values given by Riker (2) and Suit and Eardley (6) for tomato, bryophyllum, and other plants.

TABLE 4.—*Movement of crown-gall bacteria in intact internodes of sunflower*

Distance of stem fragment from point of inoculation (mm.)	Interval between inoculation and testing			
	1 hour		24 hours	
	Bacteria applied		Bacteria applied	
	At Base	At Top	At Base	At Top
5	5 (5) [a]	5 (5)	3 (3)	4 (4)
10	5 (5)	5 (5)	2 (3)	5 (5)
15	3 (3)	5 (5)	3 (3)	5 (5)
20	3 (3)	3 (3)	2 (3)	5 (5)
25	4 (4)	5 (5)	2 (2)	5 (5)
30	4 (4)	2 (4)	2 (2)	4 (5)
35	4 (4)	2 (4)	3 (3)	4 (5)
40	1 (1)	2 (5)	3 (3)	3 (4)
45	3 (3)	2 (4)	1 (1)	4 (5)
50	1 (1)	1 (4)	3 (3)	2 (3)
55	1 (1)	0 (4)	1 (1)	2 (3)
60		0 (1)		1 (2)
65				1 (1)

[a] The first figure represents the number of stem fragments yielding crown gall bacteria; the figure in parenthesis represents the number of stem fragments tested.

SUMMARY AND CONCLUSIONS

Experiments were undertaken to determine the rate of movement of crown-gall bacteria through isolated fragments of sunflower stem cultured *in vitro*.

A loopful of bacterial suspension containing between 10 and 100 organisms was applied to the upper cut surface of 9-mm. sunflower stem fragments supported on nutrient agar. Bacteria passed through the stem fragment and appeared on the agar within four minutes from the time of application. Polarity of the stem fragment did not affect the rate of movement of the bacteria.

After three days culture *in vitro*, callus formation on the cut surface of the stem fragments hindered the bacteria from passing through the stem. Both the upper and lower calluses proved effectual in this respect.

Bacteria applied to the lateral surface of isolated stem fragments placed on their sides on the medium produced tumors at the end of these fragments.

Bacteria applied to the top or base of intact sunflower internodes travelled about 5 cm. through the stem in the course of one hour. There was no significant difference in the rate of upward or downward movement.

It is concluded that the movement of crown-gall bacteria in isolated

sunflower stem fragments probably takes place through the tracheae and that the motive force is supplied by the bacteria themselves.

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SOME STUDIES OF CURLY TOP OF FLAX

N . J . G i d d i n g s [1]

Experimental infection of flax, *Linum usitatissimum* L., with curly top virus was reported by Severin[2] in 1929. In 1945 Severin and Houston[3] reported the disease from commercial fields in the central San Joaquin Valley of California and gave a brief discussion of the disease in relation to flax growing in that area.

Curly-top diseased flax was received[4] from commercial fields in that same general area in the spring of 1945 and again in 1947 and virus was recovered from these plants.

EXPERIMENTAL WORK

Experiments were begun in the spring of 1945 to determine the relative susceptibility of flax varieties to the different strains of the curly-top virus and to learn whether any of the varieties might be helpful in differentiating the virus strains. Table 1 lists the 37 flax varieties and species which have been tested. The four commercial varieties numbered 19 to 22, namely Acc. 200, Cascade, Highball, and Liral Prince, are "fiber types" and all others are seed types.

All commercial varieties of flax tested were found to be very susceptible to infection by all of the curly-top virus strains available (Fig. 1).

Young plants infected by the highly virulent curly-top virus strains 1, 3, 5, 6, 8, and 9, were dwarfed, distorted, and frequently killed, while those infected by the less virulent strains 2, 4, and 7 showed very little dwarfing and few plants dead, but distinct leaf distortion including enlarged veins, enations, rolling, and twisting as shown in figure 1. Virus strain 10, which is rated as moderately virulent on sugar beets appears to be relatively more virulent toward flax. Of 1687 young flax plants infected by the more highly virulent strains of curly-top virus, under greenhouse conditions, more than 30 per cent were dead in approximately 35 days after inoculation, while in 938 similar plants infected by the less virulent strains the mortality was only 4 per cent in the same length of time, and there was a mortality of 14 per cent among 346 plants inoculated with strain 10.

There were no significant differences in susceptibility to infection among the commercial varieties tested. Varieties 24, Linho das Indias, and 26,

Linho A. R. 81022, had appreciably less injury than others but the seed were very slow in germinating so that the inoculations were made at a different time and the supply of seed was so small that the number of plants involved was limited—12 and 20, respectively. All tests were conducted in a moderately warm greenhouse. Presumably, conditions more nearly simulating the

TABLE 1.—*Flax varieties and species tested for resistance to curly top*

River-side Number	Name or designation		Source of seed
1[a]	Rio (Argentina)	CI 280	L. G. Goar, El Centro, California
2	Calar do	CI 463	do
3	G. Sel. 1 (India)		do
4	Punjab do	CI 20	do
5	Minerva	CI 1081	J. O. Culbertson, St. Paul, Minn.
6	Rocket	CI 1133	do
7	980 × Redson	CI 1118	do
8	Koto × Redwing	CI 1095	do
9	Sheyenne	CI 1073	• do
10	Dakota	CI 1071	do
11	Redwing	CI 320	do
12	Bison	CI 389	do
13	Crystal	CI 982	do
14	Koto	CI 842	do
15	*Linum lewisii* Pursh		Theadore Payne (Seedsman)
16	*L. perenne* L.		Aggler & Musser (Seedsmen)
17	*L. flavum* L.		do
18	*L. grandiflorum* Des F. Var. *rubrum* Hort.		do
19[b]	Acc. 200		Nelson, Oregon
20	Cascade		do
21	Highball		do
22	Liral Prince		do
23	Linho caxias		• Brazil
24	Linho das Indias		do
25	Linho Farroupilha		do
26	Linho A. R. 81022		do
27	Linho Reg. 539		do
28	Minn. III–46–1		India
29	Minn. III–46–2		do
30	Minn. III–46–3		do
31	Benvenuto Labrador		Argentina
32	Buck 3		do
33	Entre Rios		do
34	Querondi M. A.		do
35	Buck 114		do
36	Klein II		do
37	La Prevision 18		do

[a] Seed for 1 through 4 were received in 1945; for all others, in 1947.
[b] Numbers 19 to 37 were all received from Dr. J. O. Culbertson and he indicated the original sources of the various lots.

environment under which flax is grown in California would be of greater value in economic evaluation as to curly-top resistance.

Plants infected by the less virulent strains of curly-top virus often show evidences of recovery similar to those reported by Wallace[5] for Turkish

[5] Wallace, J. M. Recovery from and acquired tolerance of curly-top in *Nicotiana tabacum*. Phytopath. 29: 743–749. 1939.

tobacco.   Such plants may send out a symptomless branch below the place where most pronounced symptoms appeared or they may merely continue
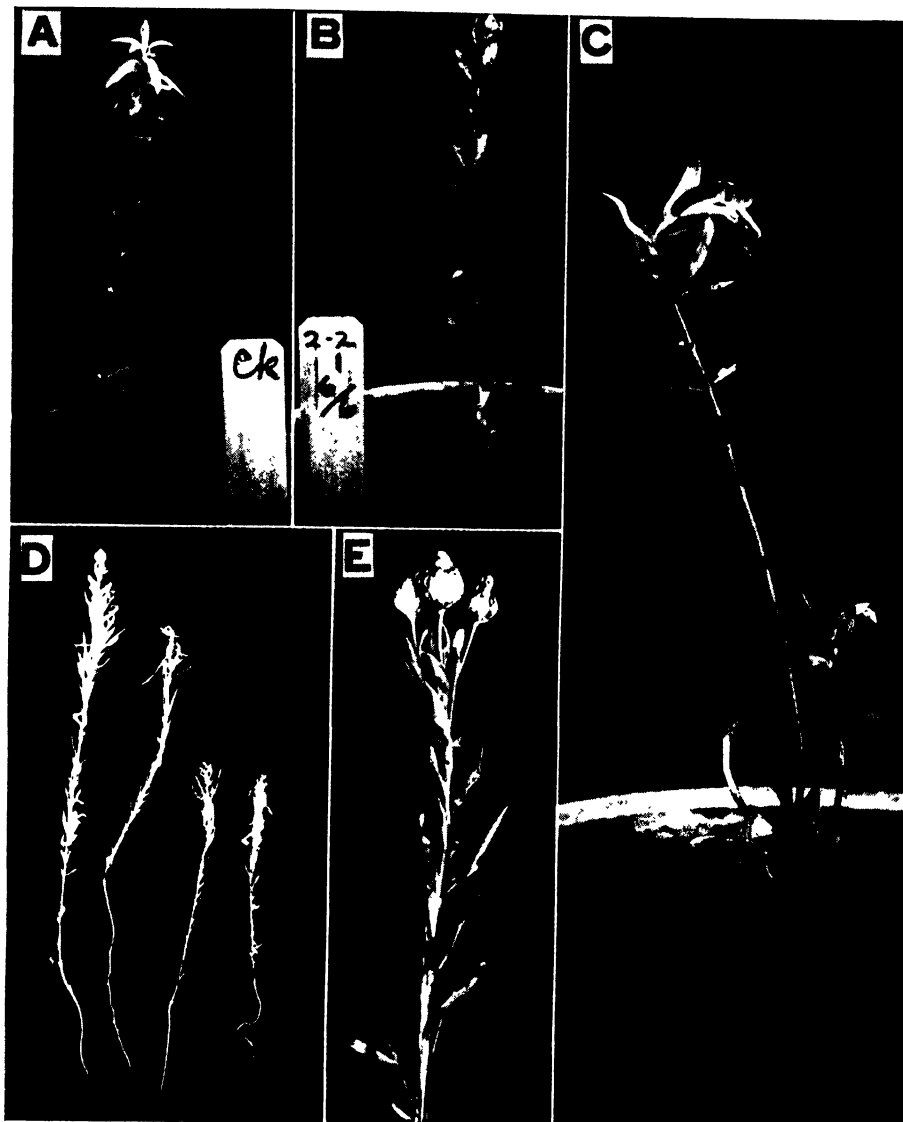
FIG. 1.   A. Healthy Rio flax plant on which non-viruliferous leafhoppers had been caged.   B. Rio plants with mild symptoms of curly top after inoculation with virus strain 2.   C. Curved tips and rugose terminal leaves of Rio flax inoculated with virus strain 8.   The enlarged, curved portion of stem is commonly light pink.   D. Dwarfed, bleached, and distorted plants infected by curly-top virus, collected from a commercial field of flax in San Joaquin Valley, California.   E. Infected plant with distorted leaves and seed pods having papillae on the sepals.

the normal terminal growth with fewer symptoms on the new growth. Symptoms may reappear in the sepals enclosing the seed pod.   Four in-

fected plants each having a symptomless branch were tested for the presence of curly-top virus. Virus was recovered from the terminal growth of each plant even though there was very little symptom-bearing tissue included in the cage of non-viruliferous leafhoppers. Virus of a concentration equivalent to that in the terminal growth was found in the symptomless branches of one plant, while another such branch gave evidence of a very low concentration of virus and no virus was obtained from symptomless branches of either of the other two plants.

Four ornamental species of flax, Nos. 15 to 18 in table 1, were also tested. One species, *Linum grandiflorum rubrum*, was fairly susceptible to infection and injury, while the other three, *L. lewisii*, *L. perenne*, and *L. flavum*, were highly resistant. These three resistant species showed no curly-top symptoms and the only evidence of infection was recovery of the virus from infected plants. The available number of plants was limited and *L. lewisii* was tested only with virus strains 1, 2, and 3. Virus was recovered from only one plant and it had been inoculated with strain 2. Plants of *L. perenne* and *L. flavum* were inoculated with virus strains 1, 2, 3, 6, 7, and 10. Tests from these plants revealed that all plants inoculated with virus strain 6 were infected, but no virus was recovered from any of the others.

DISCUSSION AND SUMMARY

The susceptibility of all commercial flax varieties tested indicates the possibility of serious injury during seasons particularly favorable for curly-top infection. Fortunately, the curly-top vector, *Eutettix tenellus* Baker, is likely to be rather inactive during cool fall and winter weather, when the flax is making most of its growth. Early planting or warm, dry weather continuing late into the fall are more favorable for curly-top infection of the young plants and consequently more crop damage.

Comparative studies of flax varieties under field conditions might reveal some differences in susceptibility which would be helpful in breeding for curly-top resistance. Some of the ornamental species have a very high degree of curly-top resistance and this offers some encouragement in the breeding for disease resistance.

There is a possibility that some of the highly resistant ornamental species may be helpful in differentiating curly-top virus strains, since infection of *L. perenne* and *L. flavum* was secured only with strain 6, but further tests are necesssary.

RIVERSIDE, CALIFORNIA

WHEATS IMMUNE FROM SOIL–BORNE MOSAIC VIRUSES IN THE FIELD, SUSCEPTIBLE WHEN INOCULATED MANUALLY[1]

H. H. McKinney

INTRODUCTION

In conducting the field tests for mosaic resistance in wheat grown in infested soils it has been found that the infestation does not always persist for long periods (2, 4, 9). Sometimes the infectivity fails in one season, whereas, in other cases it may decrease gradually or persist over a period of years with continuous wheat culture. Because of this uncertainty, it has been found advantageous to have the test nurseries located on several infested areas, thereby reducing the chances of losing a season's work.

In all of the wheat-mosaic test nurseries the author has studied east of the Mississippi River, the mosaic-rosette virus (*Marmor tritici* var. *typicum* McK.) (7) and the yellow-mosaic virus (*M. tritici* var. *fulvum* McK.) (7) have been present.[2] When the soil infestations have shown signs of decline, however, it has been observed that the mosaic rosette symptoms in Harvest Queen selection usually fail sooner than do the yellow mosaic symptoms in such varieties as Currell wheat and in Red Winter spelt. Furthermore, at Arlington Farm, Va., when Harvest Queen selection failed to manifest rosette, it failed to manifest any sign of mosaic in most of the plants, even when Red Winter spelt and yellow-mosaic susceptible wheats in adjacent rows had considerable yellow-mosaic mottling. On the other hand, in experiments with these viruses in the greenhouse, Harvest Queen selection had been used regularly for much of the inoculation with the mosaic-rosette virus and with yellow-mosaic virus, since the variety is very susceptible to both when inoculated manually. Furthermore, isolations from plants of resistant varieties manifesting mild signs of mosaic in field plats have yielded the mosaic-rosette virus.

These observations made it appear that Harvest Queen selection and the so-called mosaic-resistant wheats are less susceptible to the yellow-mosaic viruses than to the mosaic-rosette virus when grown in infested soils, and that mosaic resistance in the field is not necessarily a criterion for mosaic resistance following manual inoculation. Accordingly it was

[2] Since preparing this paper, the writer has studied a soil-borne wheat-mosaic virus occurring in the plant breeding plats of the Agricultural Experiment Station at Cornell University, Ithaca, New York. This virus has several characteristics which distinguish it from the viruses discussed in this paper.

decided to make manual-inoculation studies on some of the wheat lines that had been selected for high mosaic resistance, or were immune from mosaic when grown in the field in soils having high levels of infestation. The results of these tests have been summarized in an abstract (9).

It should be pointed out that the situation is quite different with the rosette expression. Here, there has been complete correlation between the field-grown plants and the manually inoculated plants. The great majority of wheat varieties are completely immune from rosette, apparently under all conditions of culture and of inoculation. However, most varieties are susceptible to the mosaic reaction induced by the mosaic-rosette virus.

PLANT MATERIALS

Harvest Queen selection was selected by the writer from the mass of Salzer's Prizetaker (Harvest Queen C.I. 5314). It is uniformly susceptible to mosaic-rosette in the field and also in greenhouse tests when inoculation is by manual methods.

Michigan Amber (Ind. Sel. 29–1–1–1) is uniformly susceptible to the green and the yellow mosaics, but it is immune from rosette in the field and in the greenhouse tests.

The mosaic-resistant wheats listed in table 1 develop no signs of mosaic or only weak to moderate mottling in few to many plants, depending on the season. They are uniformly immune from rosette in the field, and when inoculated by manual methods.

The field-immune lines have shown no signs of mosaic or rosette in the field, except one case to be noted. Ill. Sel. 36–686 gave no signs during about 10 years' testing on infested soil in Illinois. In 1945, however, one yellow-mosaic plant was found in two 16-foot rows in the wheat-mosaic nursery at Clemson, South Carolina. Adjacent rows of susceptible wheats had mosaic in a high percentage of the plants. It is possible that an unusual strain of virus infected this plant, but it seems more likely that the plant represented an admixture, possibly a volunteer.

The winter barley lines expressed no signs of mosaic or rosette when tested one season in the wheat-mosaic nurseries. Further information on the plant materials is given in table 1.

VIRUS COLLECTIONS

Isolates of mosaic-rosette virus (*Marmor tritici* var. *typicum*) (7) and of the yellow-mosaic virus (*M. tritici* var. *fulvum*) (7) were used. The several isolates of mosaic-rosette virus were indistinguishable, but several isolates of the yellow-mosaic virus produced consistently different symptoms on Michigan Amber wheat. All of the latter induce yellow-green to yellow markings on the foliage of Michigan Amber, and all stunt the plants. The isolates from La Porte, Indiana, and Granite City, Illinois (Table 3) are less severe than the others. The isolate from LaFayette, Indiana

(Table 4) induces extreme rolling and distortion of the leaves during their early stages of development. The isolate from Clemson, South Carolina

TABLE 1.—*Information concerning the winter wheats and winter barleys used*

Variety or selection	C.I. No.	Source of seed	Reaction to mosaic in infested soil in the field
Wheat:			
Michigan Amber 29–1–1–1 (Mildew resistant)	11770	R. M. Caldwell	Immune from rosette; very susceptible to light-green mosaic with mosaic-rosette virus; yellow mosaic with yellow-mosaic viruses.
Harvest Queen selection		H. H. McKinney	Very susceptible to rosette and light-green mosaic with mosaic-rosette virus; very resistant to or immune from yellow-mosaic viruses.
Arlando	10069	W. J. Sando	Immune from rosette; resistant to or immune from yellow mosaics; mildly susceptible to green mosaics.
Harvest Queen sel. 32–43		H. H. McKinney	Do
Ill. sel. 37–D216 from Duffy		O. T. Bonnett and B. Koehler	Do
Ill. sel. 37–D382 Fulcaster × Minturki		Do	Do
Ill. sel. 37–D544 (Minturki × Fulcaster) × Fulcaster		Do	Do
Ill. sel. 37–1146 (Kanred × Red Rock sel. H–93–2) × (Malakof × Mich. Amber)		Do	Do
Wabash	11384	R. M. Caldwell	Do
Ill. sel. 36–686 from (Gladden × Minturki) × Gladden		Bonnett and Koehler	Immune from the mosaics and rosette.
Ind. sel. 11512 from Trumbull × Fultz	12220	R. M. Caldwell and L. E. Compton	Do
Supreza × (Redhart Strain 5)		Coker's Ped. Seed Co.	Do
Barley:			
Purdue 21 (Mass sel. from Tenn. winter)	4581	Bonnett and Koehler	Do
Purdue 25 Comfort × Purdue 21		Do	Do
Purdue 28 Velvet × Purdue Acc. 1101		Do	Do
Smooth Awn 86	6268	J. W. Taylor	Do

(Table 5) does not induce leaf rolling, but it stunts the plants badly, usually causing early death in most of the population. The destruction of the plastid pigments in Michigan Amber is greatest with this isolate.

In the tests with the field-immune wheat, Trumbull × Fultz sel. 11512 (C.I. 12220), from R. M. Caldwell and L. E. Compton, the writer collected a field composite of virus from mosaic Michigan Amber plants growing immediately adjacent to each end of the nursery row of the field-immune line. This was to assure, so far as possible, the testing of this wheat against the virus population to which it had been subjected in the nursery near LaFayette, Ind. As this series of tests progressed, care was taken to get virus from the leaves of all the diseased plants of a population supplying inoculum for a subsequent test.

METHODS

All seedlings were cultured in steamed soil in 6-inch earthen pots in a cool greenhouse during the winter or in a special culture chamber during the hot months. The temperature was regulated near 60° to 65° F. In the chambers, the daily photoperiod was of 8 hours' duration.

Seedlings were inoculated when they reached the three-leaf stage. In most of these tests, the leaves of the seedlings were inoculated by drawing them between the thumb and fingers moistened with the infective juice to which 600-grain carborundum powder had been added. The leaves were then rinsed with water. When the roots or the underground leaf sheaths were inoculated, the seedlings were carefully removed from the soil or the sand, the roots were washed, and inoculation proceeded in the same manner outlined for the leaves, but without the rinsing. In one test in 1934, the inoculum was worked into the crown tissues by means of a fine needle, and the roots were bathed in the virus extract. Pouring fresh virus extracts into the soil around the seedlings failed to induce mosaic. According to Allen (1) this method of inoculation induces big vein disease (a soil-borne virus disease) in a high percentage of lettuce plants.

Inoculum was prepared from fresh mosaic leaves from moderately young wheat plants. The tissue was clipped and then thoroughly pulped with sand in a mortar, with sufficient sterile water added to make a five-fold dilution of the plant juice. The resulting fluid was passed through double surgical gauze to remove the coarser fragments of tissue and sand. The carborundum powder was added to the liquid, and the inoculation procedure was started immediately. All plants inoculated with a given virus on a given day were inoculated from the same batch of virus extract, therefore, the varietal comparisons should be confined to a given batch of inoculum.

No method of manual inoculation has yet been devised that will produce infection with the viruses under study in all of the inoculated plants in every test, even though the host is known to be homozygous for susceptibility. Throughout the investigations on these wheat viruses, many thousands of wheat seedlings have been inoculated by the leaf-wiping method, and by the pricking and root-wiping method, and it has been found

that the former usually induces the higher percentage of infection. In addition, it is much simpler and faster than the pricking and root-wiping method used in the earlier work (2, 4). Furthermore, since using this leaf-wiping method, and by giving special attention to the use of virus from moderately young succulent plants, the cultures of these viruses have shown no signs of becoming inactive, as when the earlier methods were employed (7).

<div align="center">RESULTS</div>

The results in tables 2 to 6 show that field resistance to and field immunity from mosaic in wheats is not reflected in tests where the inocula-

TABLE 2.—*Results from inoculations with Marmor tritici var. typicum (wheat mosaic-rosette virus) on winter wheats and winter barleys cultured in greenhouses or in low-temperature chambers. Virus from plants grown in soil collected near LaFayette, Indiana, spring of 1943. Inoculation was by the leaf-wiping method*

Variety or selection	Date inoculated	Seedlings	
		Inoculated	Infected[a]
		Number	*Number*	*Per cent*
Wheat:				
Michigan Amber (S)[b]	Feb. 5, 1945	29	26	89.65
Arlando (R)	Do	20	20	100.00
Ill. sel. 37–D216 (R)	Do	26	24	92.31
Ill. sel. 37–D382 (R)	Do	24	24	100.00
Ill. sel. 37–D544 (R)	Do	33	29	87.88
Ill. sel. 37–1146 (R)	Do	28	25	89.28
Michigan Amber (S)	May 19, 1945	63	25	39.68
Ill. sel. 36–686 (I)	Do	51	26	50.98
Barley:				
Purdue 21 (I)	Feb. 5, 1945	32	5	15.62
Purdue 25 Hybrid (I)	Do	22	1	4.54
Purdue 28 Hybrid (I)	Do	18	0	0
Smooth Awn 86 (I)	Do	29	0	0

[a] In all varieties and lines, the symptoms consisted of light-green mosaic mottling, but they tended to be strongest in Ill. sel. 36–686, the only field-immune wheat in this series of tests.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

tion is by manual methods. With all of the virus collections used, the mosaic symptoms were as strong in wheats that are resistant or immune in the field as they were in the susceptible controls. In one case (Table 2), a field-immune wheat gave a stronger mosaic reaction than obtained in the susceptible control.

When grown in the infested nurseries, all of the resistant lines and varieties manifest mild to moderate mosaic symptoms in few to many plants during some seasons, whereas they may show no signs during other seasons, even though the susceptible controls have much mosaic, much of it being yellow mosaic. In the present tests with manual inoculation, only ᵗhe

mild mosaic developed when the mosaic-rosette virus was used (Table 2). When the yellow-mosaic virus isolates were used, the field-resistant lines developed yellow mosaic, just as in the field-susceptible Michigan Amber controls (Tables 3 to 5). The degrees of stunting and leaf malformation, however, varied among the several wheat lines.

When comparisons are based on the percentage of the inoculated seedlings developing mosaic after the first inoculation, there is little indication of varietal difference in resistance. In tables 2 to 5 it will be observed that most of the tests show the higher percentages of mosaic plants in the field-resistant and field-immune lines. This may be accounted for by the

TABLE 3.—*Results from inoculations with Marmor tritici var. fulvum (Prairie wheat yellow-mosaic virus) on winter wheat cultured in greenhouses. Virus isolate used in 1934, collected near Granite City, Illinois, and isolate used in 1946, collected near La Porte, Indiana. Inoculation was by the leaf-wiping method, except as indicated*

Variety or selection	Date inoculated	Virus dilution	Seedlings	
			Inoculated	Infected[a]
			Number	*Number*	*Per cent*
Harvest Queen sel. (R)[c]	Mar. 13, 1934	5 ×	36	23	63.89[b]
Harvest Queen sel. 32–43 (R)	Do	Do	24	19	79.17[b]
Michigan Amber (S)	Apr. 5, 1946	Do	9	7	77.78
Ill. sel. 36–686 (I)	Do	Do	9	7	77.78
Michigan Amber (S)	Do	250 ×	31	9	29.03
Ill. sel. 36–686 (I)	Do	Do	31	9	29.03
Michigan Amber (S)	Feb. 10, 1947	5 ×	48	48	100.00
Ind. sel. 11512 (I)	Do	Do	48	47	97.92

[a] In all varieties and lines the symptoms consisted of mild to severe yellow mosaic mottling.

[b] Inoculation was accomplished by removing the seedlings from the soil, dipping the roots and crowns in fresh inoculum, and pricking the inoculum into the crown and leaf sheaths with a fine needle.

[c] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

fact that there is a small proportion of Michigan Amber susceptible controls in these tests. In table 6, where more Michigan Amber controls were tested, the differences are more in favor of the resistant and immune lines, but they are slight when compared with the differences that obtain in the field. In all of the tests (Tables 2 to 6), there is a total of 32 comparisons that may be made between the susceptible control and the resistant and immune wheats. Of these comparisons, 14 show more, and 14 show fewer mosaic plants among the field-resistant and field-immune wheats. Four of the comparisons are equal.

With the composite collection of virus (Table 6), there are 7 comparisons, 2 of which show more, and 5 show fewer mosaic plants among the field-immune lines. Taking the totals from all the tests in table 6, Michigan Amber susceptible control developed mosaic in 42.71 per cent of the

seedlings after the first inoculation, whereas, the field-immune wheats developed mosaic in 33.85 per cent of the seedlings. This drop of 8.86 per

TABLE 4.—*Results from inoculations with the leaf-distorting strain of Marmor tritici var. fulvum (Prairie wheat yellow-mosaic virus) on winter wheats and winter barleys cultured in greenhouses. Virus from plants grown in soil collected near Lafayette, Ind., in 1939. Inoculation was by the leaf-wiping method, except as indicated in the footnote*[d]

Variety or selection	Date of test	Virus dilution	Seedlings					
			Inoculated	Infected[a]		Re-inoculated	Infected	
			No.	No.	Pct.	No.	No.	Pct.
Wheat:	Jan. 11,							
. Michigan Amber (S)[b]	1945	5×	27	16	59.26	11	11	100.00
Arlando (R)	Do	Do	20	19	95.00	1	1	100.00
Ill. sel. 37–D216 (R)	Do	Do	26	19	73.08	7	7	100.00
Ill. sel. 37–D382 (R)	Do	Do	27	26	96.30	1c	0	96.30
Ill. sel. 37–D544 (R)	Do	Do	29	23	79.31	6	6	100.00
Ill. sel. 37–1146 (R)	Do	Do	28	28	100.00	..	. ..	. ....
	Feb. 6,							
Michigan Amber (S)	1946	Do	50[d]	0	0.00			
Ill. sel. 36–686 (I)	Do	Do	50[d]	0	0.00	. .		
	Feb. 25,							
Michigan Amber (S)	1946	Do	43	25	58.14	.		
Wabash (R)	Do	Do	50	19	38.00	.		. .
	Apr. 5,							
Michigan Amber (S)	1946	Do	9	9	100.00	.		
Ill. sel. 36–686 (I)	Do	Do	8	6	75.00	..		.
Michigan Amber (S)	Do	250×	36	21	58.33	.	. . .
Ill. sel. 36–686 (I)	Do	Do	28	13	46.43	.	.. ..
	Jan. 17,							
Michigan Amber (S)	1947	50×	30	30	100.00	.		
Ill. sel. 36 686 (I)	Do	Do	56	56	100.00		. .
	Feb. 10,							
Michigan Amber (S)	1947	5×	48	42	87.50	.	. ..
Ind. sel. 11512 (I)	Do	Do	48	44	91.67			
	Feb. 25,							
Michigan Amber (S)	1947	Do	24	24	100.00			
Ill. sel. 37–D382 (R)	Do	Do	26	24	92.31	2	2	100.00
Barley:	Jan. 11,							
Purdue 21 (I)	1945	Do	28	1	3.57	27	1	7.14
Purdue 25 (I)	Do	Do	21	0	0.00	21	0	0.00
Purdue 28 (I)	Do	Do	28	1	3.57	27	0	3.57
Smooth Awn (I)	Do	Do	29	1	3.45	28	6	24.14

[a] In all varieties and lines, the symptoms consisted of mild to severe yellow-mosaic mottling.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

[c] This plant was inoculated a third time and failed to become infected. It was not inoculated again as resistance tends to increase with age, but it was isolated from other wheat plants, and allowed to set seed. This seed was sown, and the 26 seedlings were inoculated Feb. 25, 1947, with the results shown in the last item under wheat in this table.

[d] Inoculation was accomplished by removing the seedlings from the soil, and wiping the underground portion of the first leaf sheath and the roots with fresh virus extract and carborundum powder.

cent in the incidence of mosaic is not very great when we take into account that these wheats have been immune in the fields.

It was not practicable to reinoculate all of the seedlings that escaped infection after the first inoculation. This was done, however, in a few tests shown in tables 4 and 6. In some of these tests, all of the escapes developed mosaic after the second inoculation. In table 4, one plant of Ill. sel 37–D382 failed to show mosaic after being inoculated three times. However, when the progeny (26 seedlings) from this plant was inoculated, 24 seedlings developed yellow mosaic from the first inoculation and the remaining two after a second inoculation. In table 6 it will be noted that a fairly large percentage of the seedlings remained mosaic-free after the second inoculation.

When the composite virus collection was used (Table 6), the symptoms consisted of a mixture of green and of yellow mosaic, and as the tests pro-

TABLE 5.—*Results from inoculations with Marmor tritici var. fulvum (Prairie wheat yellow-mosaic virus) on winter wheat cultured in greenhouses. Virus collected near Clemson, South Carolina. Inoculation was by the leaf-wiping method*

Variety or selection	Date of test	Virus dilution	Seedlings		
			Inocu-lated	Infected[a]	
			Number	*Number*	*Per cent*
Michigan Amber (S)[b]	Apr. 5, 1946	5 ×	10	3	30.00
Ill. sel. 36–686 (I)	Do	Do	9	3	33.33
Michigan Amber (S)	Do	250 ×	33	7	21.21
Ill. sel. 36–686 (I)	Do	Do	33	7	21.21
Michigan Amber (S)	Dec. 30, 1946	50 ×	48	47	97.92
Supreza × (Redhart Str. 5) (I)	Do	Do	45	43	95.55
Michigan Amber (S)	Jan. 17, 1947	Do	30	30	100.00
Supreza × (Redhart Str. 5) (I)	Do	Do	51	49	96.08

     [a] In all varieties and lines the symptoms consisted of moderate to severe yellow-mosaic mottling.
     [b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

gressed, the proportion of green mosaic tended to increase very slightly, suggesting that unilateral interference was operating very weakly in the culture. All observations to date support the view that if unilateral interference does occur among the viruses that have been isolated from wheat plants that contracted mosaic by way of the soil, it is not a striking phenomenon (3, 7). Weak unilateral interference (antagonism) was reported by the writer (5) in three mutants from tobacco-mosaic virus, but in these cases, each of the two yellow-mosaic mutants dominated the mild-green mosaic mutant.

Facilities have not permitted a thorough study of virus isolates from the mild-mosaic wheat plants that occur in the resistant lines in the field, but the few isolations that have been made indicate that some of these plants are infected with the mosaic-rosette virus. Also, it appears that

non-rosetting green-mosaic viruses are present in some areas, i.e., viruses that will not induce the rosette reaction in Harvest Queen selection.

In tables 2 and 4 it will be noted that very few plants of the field-immune barley lines developed mosaic in the manual-inoculation tests

TABLE 6.—*Results from inoculations with a field composite collection of strains of Marmor tritici on winter wheats cultured in greenhouses and low-temperature culture chambers. The virus culture was isolated from mosaic Michigan Amber plants growing near Lafayette, Ind., in nursery rows adjacent to each end of a row of Ind. sel. 11512 that developed no mosaic in the field. Inoculation was by the leaf-wiping method, except as indicated in the footnotes*

Variety or selection	Date of test	Virus dilu-tion	Seedlings					
			Inocu-lated	Infected[a]		Re-in-ocu-lated	Infected	
			No.	No.	Pct.	No.	No.	Pct.
Michigan Amber (S)[b]	June 21, 1946	5×	46	30	65.22	...	.	.
Ind. sel. 11512 (I)	Do	Do	50	33	66.00	.	.	...
Michigan Amber (S)	Aug. 15, 1946	Do	80	16	20.00		.	.
Ind. sel. 11512 (I)	Do	Do	80	10	12.50			.
Michigan Amber (S)	Oct. 21, 1946	Do	40	21	52.50	.	..	. . ..
Ind. sel. 11512 (I)	Do	Do	40	12	30.00			
Michigan Amber (S)	Dec. 2, 1946	50×	40	13	32.50	27	16	72.50
Ind. sel. 11512 (I)	Do	Do	40	3	7.50	37	10	32.50
Michigan Amber (S)	Dec. 30, 1946	Do	58	30	51.72	28	6	62.07
Ind. sel. 11512 (I)	Do	Do	61	40	65.57	21	7	77.05
Ill. sel. 36–686 (I)	Do	Do	66	31	46.97	35	9	60.61
Michigan Amber (S)	Jan. 17, 1947	Do	24	7	29.17	.		
Ind. sel. 11512 (I)	Do	Do	59	5	8.47	...		
Michigan Amber (S)	Jan. 28, 1947	5×	53	18	33.96	.. .	.	
Do	Do	Do	40c	3	7.50	.		
Do	Do	Do	40d	2	5.00		. .	
Ind. sel. 11512 (I)	Do	Do	40c	1	2.50		. . ..	
Do	Do	Do	40d	0	0.00			

[a] In each test, the mosaic mottling was essentially of the same degree in the field-immune wheat as in Michigan Amber, the field-susceptible wheat.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (S) Susceptible, (I) Immune.

[c] Inoculation was accomplished by removing seedlings from the soil and wiping the underground portion of the first leaf sheath with fresh virus extract and carborundum powder.

[d] Inoculation as above, except that the roots only were wiped.

Comparatively few varieties of barley have been tested in the mosaic fields, but the tests that have been conducted suggest that barley tends to be more resistant than wheat. However, Nakano Wase barley is extremely susceptible to mosaic in the field.

CONCLUSIONS

When grown in virus-infested soils, it appears that the field-resistant wheats may be resistant in varying degrees to one or more of the green mosaics, and immune, in most cases, from the yellow mosaics. Whereas, the field-immune wheats appear to be immune from both green and yellow mosaics. The breakdown of immunity from mosaic following inoculations by manual methods cannot be explained at present. It is possible that a more delicate method of manual inoculation might reflect the same varietal differences in mosaic susceptibility as obtained in the field. This does not seem to be a very promising approach, however, at present. It may be that none of the infested soils encountered thus far contain sufficient amounts of active virus to induce yellow mosaics in the field-immune wheats. If it were possible to increase virus activity or concentration in the soil, this point could be tested, but all attempts to infest soil by adding mosaic leaf tissue or fresh virus extracts have failed. The field differences in susceptibility might be reflected if the manual inoculations were made on older plants which are more resistant than the seedlings and young plants. It is possible that immunity in the field is against a vector of some sort, rather than directly against virus. If this is the case, more than one vector may be involved, and there may be a hitherto unsuspected, strong interference between the yellow-mosaic and the green-mosaic viruses when present in certain genotypes of wheats. Otherwise, it is difficult to understand how one group of wheats can be susceptible to green mosaics and immune from yellow mosaics, whereas another group growing in the same immediate area in a field will be immune from both green mosaic and yellow mosaic.

It seems likely that the range of reaction shown among the virus isolates used in these studies will be extended as new isolates are studied, especially isolates from plants out of the field-resistant group. It is already known that the mosaic-rosette virus induces a moderately yellow mosaic in Red Winter spelt (3, 4), whereas, in all of the wheats tested against this virus in the present studies, only light-green mosaics occurred. Whether a study of new isolates will shed further light on the problems covered by the present tests, is a question. The wheats and the virus isolates used in the tests were chosen at random, yet all of the wheats reacted alike, within narrow limits, rosette excepted, when inoculated with any one of the several virus isolates used.

With the tobacco-mosaic virus it is possible to make rapid progress in the resistance studies with tobacco (6, 8) and other crops when manual methods of inoculation are used, but it is evident from the results presented, that this is not true in the case of the soil-borne mosaic viruses in wheat. Here the routine testing and the genetic studies must be done in the infested soil, except in the case of the rosette reaction, which can be studied when the inoculations are done manually. With the mosaic reactions, precise genetic studies will be difficult, even when closely coordi-

nated with studies on the several isolates which make up the virus population of the test soil, because there is no way at present to test the virus isolates separately by nature's method of inoculation.

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DIP AND GAS TREATMENTS FOR THE REDUCTION OF POST-HARVEST DECAY IN TEXAS LEMONS

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A previous publication[3] described the effects of wrappers impregnated with diphenyl, chemical dips, and stem swab treatments for the control of decay in lemons after harvest. Many chemicals used as dips were fairly effective in reducing Diplodia and Penicillium rots. However, none of the materials that were considered to be safe from the standpoint of fruit injury and chemical residues were sufficiently fungicidal to ensure that the fruit would be commercially acceptable after the usual transit and marketing period.

A gaseous mixture of nitrogen trichloride and air has been reported by Klotz[4] to be effective in controlling *Penicillium italicum* (blue mold) and *P. digitatum* (green mold) decay of Washington Navel and Valencia oranges in California. Its effectiveness for control of stem-end decay (*Diplodia natalensis*) was not known.

As Texas lemons are commonly placed in a coloring (degreening) room for treatment with ethylene gas for one day to 6 or 7 days, depending upon harvest maturity, it was felt that the coloring room would be the logical place to treat the fruit with a fungicidal gas. Since certain chemicals had been demonstrated to be at least partially effective as dips before coloring, the experiments were set up in such a way that results could be obtained on dips alone, on gas alone, and on a combination of the dip and gas treatments.

METHODS

Eureka lemons were harvested early in October, late in October, and in December and January. As soon as the fruit was brought in from the field, composite samples of 200 fruits each were selected by taking 10 to 12 fruits of normal size and appearance from each of 17 to 20 field boxes. Injured or overripe fruits were discarded. Dipping was done as soon as sampling was completed, then all were placed in the coloring rooms. Fruits in one room received the regular commercial coloring treatment with ethylene gas. Fruits in the other received ethylene interrupted at intervals for the introduction of the mixture of NCl_3 and air. The first treatment with NCl_3 always was applied in the evening of the day the fruit was harvested and

within a few hours of the time the fruit was placed in the coloring room. Four hours was the usual duration of treatment and the concentration of NCl_3 varied from 0.003 to 0.04 p.p.m. Additional treatments with NCl_3 were made at intervals of 24 to 48 hours.

The lemons were held in the coloring room for 8 days in early October but only 2 days in January, the time depending upon the degree of coloration when the fruits were harvested. At the completion of coloring they were removed from the room, examined for decay, wrapped in regular tissue wrappers, and packed in standard boxes which were held in a non-refrigerated storeroom in the warehouse at temperatures that varied from 75° to 85° F. in October and from 50° to 75° F. in January.

Each lot was inspected for decay one week after packing, after which it was repacked and held for an additional week to ten days. The final inspection was made 20 to 22 days after harvest.

<center>RESULTS</center>

In lemons harvested early in October stem-end rot was responsible for all of the losses during the holding period (Fig. 1,A). The lot that received no dip after harvest and no nitrogen trichloride during degreening averaged over 50 per cent decay 1 week after packing and 70 per cent decay 2 weeks after packing. The lot that received no dip but was treated with a very low concentration (0.003 p.p.m.) of NCl_3 in the coloring room had very little decay 1 week after packing but 13.5 per cent in the second week. A dip with the quaternary ammonium compound "Adbac" had very little effectiveness at the concentration used, but when this dip was followed by treatment with NCl_3 the stem-end rot was only 2 per cent 2 weeks after packing.

In lemons picked in late October, stem-end rot was responsible for all of the decay found during the holding period (Fig. 1,B). One week after packing, the untreated lot of Eureka lemons had 64 per cent decay and the similar lot of Meyer lemons, a little less than 30 per cent. A slightly higher concentration of NCl_3 (0.005 p.p.m. as Cl_2) than that used in the first test gave only fair control when used on non-dipped fruit. Neither the Adbac dip nor the thiourea dip, used without subsequent NCl_3 treatment, was adequate for control although both substantially reduced decay as compared with no dip check.

By the time the third experimental lot was picked (Dec. 9) the blue and green molds were becoming a factor in total decay, although stem-end rot still predominated, particularly in the non-gassed lots (Fig. 1,C). In this test the concentration of NCl_3 was increased to 0.03 p.p.m. and the results indicate that the gas was more effective at this strength than at lower concentration. The untreated check had 22 per cent stem-end rot and 4 per cent blue and green mold rot, or a total of 26 per cent decay, 17 days after packing, whereas the non-dipped but NCl_3 treated fruit had 2 per cent stem-end rot and 1.5 per cent green mold rot, or a total of 3.5 per cent decay, after the same period.

With lemons harvested early in January, stem-end rot was of minor importance and the predominating decay was green mold (Fig. 1,D). The untreated check lot had over 29 per cent blue and green mold rot and less
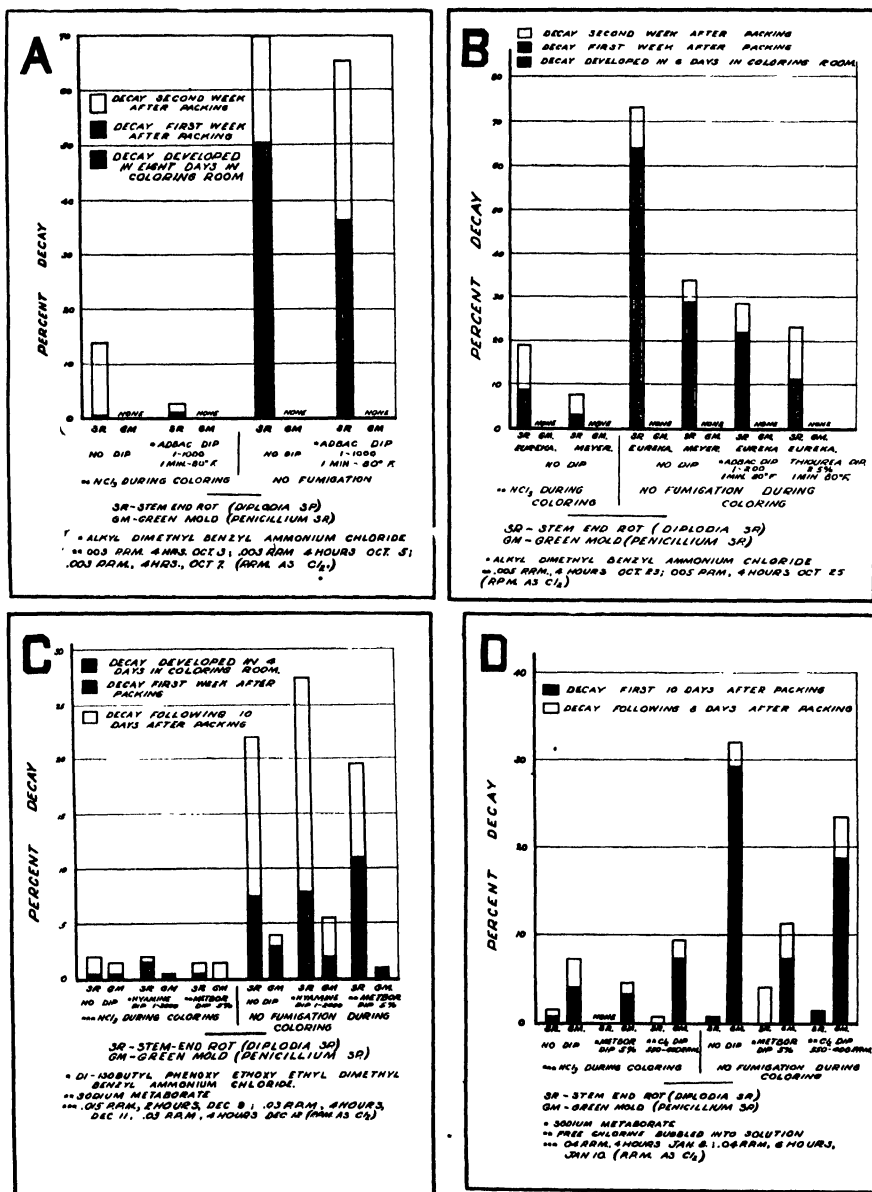
FIG. 1. The control of decay in lemons after harvest as influenced by NCl₃ gas and dip treatments: A. Lemons harvested Oct. 3, 1946; B. Harvested Oct. 23, 1946; C. Harvested Dec. 9, 1946; D. Harvested January 8, 1947.

than 1 per cent stem-end rot 10 days after packing. Comparable lots treated with NCl₃ at 0.04 p.p.m. during degreening developed 4 per cent

green mold rot and about 0.5 per cent stem-end rot. Neither sodium metaborate nor chlorine-solution dip was satisfactory for control of green mold rot when such dips were the only treatments used. Results were better when both dips were followed by NCl₃; but the chlorine solution dip followed by NCl₃ had no advantage over the gas treatment alone.

DISCUSSION

Treatment of lemons with nitrogen trichloride at concentrations of 0.003 to 0.04 p.p.m. for several periods of approximately 4 hours each during the degreening process was responsible for a significant reduction in the amount of stem-end rot and blue and green mold rot which developed during the holding period after packing.

No evidence of injury to lemons was found as a result of treatment with NCl_3 at the concentrations used, and the interruptions in the ethylene gas treatment for the purpose of treating with NCl₃ appeared to have no significant effect upon the rate of degreening.

The quaternary ammonium compounds tested were of little promise when used alone; but when used as post-harvest dips followed by nitrogen trichloride treatment, both compounds gave results somewhat better than were obtained with the gas treatment alone. Sodium metaborate solution alone was partially effective for the control of both stem-end and green mold rots. However, when sodium metaborate was used in combination with NCl_3 gas the control of rot was better than with the dip alone or the gas alone. None of the solutions used as dips had any visibly adverse effects upon the lemons either when used alone or when used in combination with nitrogen trichloride. A slight bleaching resulted from the use of a dilute chlorine solution, but this added to rather than detracted from the appearance of the lemons.

There were seasonal differences in the type of decay: stem-end decay was completely dominant in October, some Penicillium decay appeared in December, and in January the green mold was predominant and there was little stem-end decay. This changing relationship undoubtedly is closely related to temperatures in the grove and in the warehouse after packing. Diplodia stem-end decay requires relatively high temperatures for optimum development, whereas green mold and blue mold fungi thrive at considerably lower temperatures. Outdoor temperatures in October averaged about 78° F., whereas in early January they averaged about 60° F.

SUMMARY

Certain chemical solutions as post-harvest dips and the use of nitrogen trichloride gas in the degreening room are discussed in connection with the handling of Eureka and Meyer lemons.

Nitrogen trichloride, in concentrations ranging from 0.003 to 0.04 p.p.m., when applied for 2 to 4 periods of approximately 4 hours each during degreening reduced stem-end and green mold rots materially.

Two quaternary ammonium compounds, sodium metaborate, and chlorine solutions were of only fair fungicidal value when used as dips at the concentrations tested; but when combined with nitrogen trichloride gas treatments in the coloring room they controlled both stem-end and green mold rots.

The relative importance of stem-end rot and green mold rot changes during the season, stem-end rot being dominant in October and green mold rot of major importance in January.

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A COMPARISON OF FUNGICIDAL TREATMENTS FOR THE CONTROL OF DECAY IN CALIFORNIA CANTALOUPES

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Decays of western-grown cantaloupes are not infrequently observed in shipments arriving on eastern terminal markets or during the several-day marketing period following unloading. When refrigeration is inadequate, the transit period abnormally long, or the cantaloupes unusually ripe, decay is more prevalent. The decays most commonly observed are rhizopus rot, cladosporium rot, alternaria rot, and fusarium rot.[2]

Tests were begun in 1943 and continued at irregular intervals through 1946, to determine whether some of the fungicidal treatments used with other fruits and vegetables might have value in controlling cantaloupe decays.

The studies included: (1) storage tests at Fresno, California, (2) shipping tests from Fresno (or the vicinity thereof) to New York City, and (3) laboratory tests with nitrogen trichloride at New York City.

MATERIALS AND METHODS

The following treatments were tried:

Gas treatments. Ozone, carbon dioxide, sulfur dioxide, nitrogen trichloride.

Washes. Sodium tetraborate, sodium metaborate, boric acid, sodium hypochlorite, calcium hypochlorite, merthiolate, quaternary ammonium compounds.

Dusts. Sulfur, sodium bisulphite.

Waxes. Carnauba-paraffin wax emulsion, wax-sodium bisulphite mixture.

Wraps. Diphenyl, copper-oxyquinoline.

Treated ice. Ice containing benzoic acid or chloramine.

The materials were tested by treating fairly large samples of melons, relying upon natural infection and repeated tests to establish their effectiveness. After treatment the melons in the storage tests were held at temperatures of 50° F. or above to promote the growth of the fungi causing decay. In all tests, fully mature "choice" or "hard-ripe" melons of the Powdery Mildew Resistant No. 45 variety were used.

[2] Wiant, James S. Investigations of the market diseases of cantaloupes and honey dew and honey ball melons. U. S. Dept. Agr. Tech. Bul. 573. 1937.

STORAGE TESTS

In a preliminary test in 1943, cantaloupes were exposed to ozone in concentrations of 20 p.p.m. up to nine hours at 70° F. The treatment was ineffective in controlling subsequent decay in spite of the high concentration used. These results were verified in later tests (Table 1, Test 1) when ozone

TABLE 1.—*Comparison of fungicidal treatments for the control of decay in cantaloupes, 1943 season*

Treatment	No. of melons	Percentage of melons decayed
Test 1. Sept. 1–13; stored 7 days at 65°, and 5 days at 50° F.		
Untreated	45	80
CO₂ storageᵃ	42	90
Ozone fumigation, 20 p.p.m., 8 hr.	56	79
Sulfur dust	45	62
Sodium bisulphite dust	45	36
Test 2. Sept. 14–30; stored 14 days, slow cooling 70° to 55° F.		
Untreated	45	91
CO₂ storageᵇ	13	77
SO₂ fumigation, 0.25 per cent, 20 min.	45	76
Sodium bisulphite dust	26	73
SO₂ fumigation, 1.0 per cent, 20 min.	44	68
Wax coating (Brogdex B-123)	27	52
Sodium hypochlorite wash, 0.2 per cent Cl₂, 2 min.	14	21
Wax-bisulphite coatingᶜ	26	11
Borax wash, 2½ per cent, 100° F., 2 min.	12	0
Test 3. Oct. 1–18; stored 14 days (1 day 70°, 3 days 65°, 10 days 50° F.)		
Untreated	30	80
Sodium hypochlorite wash, 0.2 per cent Cl₂, ½ min.	30	44
Borax wash, 2½ per cent, 75°, ½ min., rinsed	30	40
Borax wash, 2½ per cent, 110°, ½ min., rinsed	30	20
Borax wash, 2½ per cent, 75°, ½ min., not rinsed	30	7
Borax wash, 2½ per cent, 110°, ½ min., not rinsed	30	0

ᵃ Refrigerator car concentrations: 17–30 per cent first 17 hours, 6–8 per cent following four days.
ᵇ Refrigerator car concentrations: 20–28 per cent first 17 hours, 6 per cent five days.
ᶜ Wax and sodium bisulphite in proportion of 75 gm.: 35 gm.

was compared with sulfur dust, sodium bisulphite dust, and carbon dioxide atmospheres comparable to those obtained in refrigerator cars in cherry shipments.[3] Sodium bisulphite dust was the best of these treatments in 1943, but other materials were superior to it in test 2 (Table 1) when it was compared with sulfur dioxide fumigation, borax and hypochlorite washes, carbon dioxide atmospheres, and waxes. In test 2, borax gave perfect control of decay for the 14-day holding period. The wax-bisulphite mixture was next in effectiveness, followed by sodium hypochlorite. The other treatments were unpromising in comparison with these three. Fumigation with

[3] Gerhardt, Fisk. A study of CO₂ gas retention in refrigerator cars during the commercial freight shipment of sweet cherries. H. T. & S. Office Rept. 75, U. S. Dept. Agr., Bur. Plant Ind., Soils, and Agr. Engin. 1941.

sulfur dioxide caused no injury of any consequence even in one per cent concentration, but it was of little value in controlling decay. The wax-bisulphite treatment, omitted in later tests, resulted in an unsightly deposit of wax on the melons owing to break down of the emulsion; there was also some browning of the stem scars. Additional tests with borax (Test 3, Table 1) demonstrated that warming the solution enhanced its fungicidal action, especially when it was not rinsed from the melons. Similar conclusions were drawn from work done on the control of citrus rots with borax washes.[4]

TABLE 2.—*Comparison of fungicidal treatments for the control of decay in cantaloupes, 1944 and 1946*

Treatment	No. of melons	Percentage of melons decayed
Test 4. Aug. 8–17, 1944; stored 6 days at 65° F.		
Untreated		
Wrapped, untreated paper	60	73
Wraps, containing 1.5 per cent Cu., 1.2 per cent oxyquinoline, 11 per cent mineral oil	60	87
	60	88
Diphenyl wraps	60	73
NCl$_3$ fumigation, 5 hr., 11 mg. per cu. ft.	72	32
Sodium hypochlorite wash, 1.0 per cent Cl$_2$, 2 min.	60	8
Merthiolate wash, 1: 20,000, 2 min.	60	2
Borax wash, 5 per cent, 100° F., 2 min.	60	0
Test 5. Aug. 17–26, 1944; stored 6 days at 65° F.		
Untreated	60	58
Sodium bisulphite wash, 2.0 per cent SO$_2$, 2 min.	60	42
NCl$_3$ fumigation, 4 hr., 15 mg. per cu. ft.	60	32
Sodium hypochlorite wash, 0.75 per cent Cl$_2$, 2 min.	60	20
Merthiolate wash, 1: 20,000, 2 min.	60	20
Wash, borax 2½ per cent, boric acid 2½ per cent, 75° F., 2 min.	60	8
Borax wash, 5 per cent, 100° F., 2 min.	60	2
Test 6. Sept. 17–27, 1946; stored 10 days at 50° F.		
Untreated	60	42
No-bac ice covering[a]	60	33
Cetyl trimethyl ammonium bromide wash, 1: 1,000, 2 min.	60	18
Phenolated quaternary wash, 1: 1,000, 2 min.	60	15
Lauryl dimethyl benzyl ammonium chloride wash, 1: 1,000, 2 min.	60	7
Calcium hypochlorite wash, ¼ per cent Cl$_2$, 2 min.	60	7

[a] Contained benzoic acid and chloramine in unknown quantities, melons covered with crushed ice three days.

Sodium hypochlorite was also used in test 3 but it was not so effective in controlling decay as borax solutions.

The tests were continued the following season using stronger concentrations of borax and of hypochlorite as well as other materials. A warm 5 per cent borax solution again gave perfect control of decay (Test 4, Table 2); next in order were merthiolate (1: 20,000 dilution), and sodium hypochlorite washes. Diphenyl wraps and copper-oxyquinoline wraps were of no ap-

[4] Winston, J. R.   Reducing decay in citrus fruits with borax.   U. S. Dept. Agr. Tech. Bul. 488. 1935.

preciable value. Nitrogen trichloride fumigation reduced subsequent decay to about half that in the untreated melons. These four effective treatments and a sulfurous acid solution made with sodium bisulphite were compared in another test. A warm solution of borax gave the best control, followed in effectiveness by a borax-boric acid mixture, merthiolate, sodium hypochlorite, and nitrogen trichloride (Test 5, Table 2). Other materials, including calcium hypochlorite, quaternary ammonium compounds, and a bactericidal ice, were tried in test 6 (Table 2). Calcium hypochlorite and one of the quarternary compounds gave good control of decay during a 10-day period. Covering the melons with bactericidal ice, as used commercially on fish, was of little value against cantaloupe rots.

<div align="center">SHIPPING TESTS</div>

At the end of the second season's trials (1944) a test shipment of treated cantaloupes was sent to New York as part of a commercial carload of melons.

TABLE 3.—*Decay development in test shipments from California to New York, New York*

Treatment[a]	No. of melons	Percentage of melons decayed
Shipment 1. Aug. 25–Sept. 5, 1944; 12 days in transit, 4 days on market		
Untreated	89	66
Borax wash, 5 per cent, 100° F., 2 min.	63	25
Borax wash, nitrogen trichloride fumigation, 5 hr., 17 mg. per cu. ft.	90	24
Nitrogen trichloride fumigation, 5 hr., 17 mg. per cu. ft.	89	24
Shipment 2. Aug. 28–Sept. 9, 1945; 12 days in transit, 2 days on market		
Untreated	59	42
Sodium metaborate wash, 5 per cent, 78° F., 2 min.	61	10
Calcium hypochlorite wash, 0.3 per cent Cl_2, 78° F., 2 min.	60	9
Nitrogen trichloride fumigation, 6 hr., 25 mg. per cu. ft.	63	8
Shipment 3. Aug. 28–Sept. 9, 1945; 12 days in transit, 3 days on market		
Untreated	51	31
Calcium hypochlorite wash, 0.3 per cent Cl_2, 78° F., 2 min.	46	20
Nitrogen trichloride fumigation, 6 hr., 25 mg. per cu. ft.	52	10
Sodium metaborate wash, 5 per cent, 78° F., 2 min.	54	4

[a] Washes applied prior to packing of Shipment 1; Washes applied to packed crates of Shipment 2 and 3. Fumigation after packing and loading.

A warm borax wash for melons prior to packing was compared with nitrogen trichloride fumigation and with a combination of both treatments. All the treatments gave some control of decay and were about equal in effectiveness (Shipment 1, Table 3). The following season sodium metaborate (more soluble in cold water than borax) and calcium hypochlorite solutions used as dips for packed crates of melons were compared with nitrogen trichloride

fumigation in two shipments to New York. In one of the test cars all three treatments gave about the same control (Shipment 2, Table 3); in the other, sodium metaborate was the best of the three treatments (Shipment 3, Table 3).

TOLERANCE OF CANTALOUPE PATHOGENS TO NITROGEN TRICHLORIDE

In earlier tests on cantaloupes, nitrogen trichloride fumigation gave rather erratic results.[5] Concentrations employed were 5 to 10 mg. per cubic foot.[6] In one test when 12 mg. per cubic foot was tried, better control was obtained. In the trials reported here the concentrations were 11, 15, 17, and 25 mg. per cubic foot and rather consistent reduction in decay was observed.

Tolerance tests with NCl_3 on cultures and viable spores of cantaloupe pathogens were made using methods described by Klotz.[7] Higher concentrations were required to kill spores of the fungi on dried filter paper plates (some mycelium no doubt was included in the transfer) than cultures 1 to 4 days old on agar plates. Five hours' exposure to concentrations of 15 to 18 mg. NCl_3 per cu. ft. failed to give complete kill of spores of any of the fungi, which included *Alternaria* sp., two strains of *Rhizopus* sp., *Cladosporium* sp., *Fusarium* sp., and *Botrytis* sp. Higher concentrations of 20 to 22 mg. per cu. ft. were lethal except to one of the strains of *Rhizopus*. In another test, 21 to 22 mg. per cu. ft. failed to kill all spores of *Alternaria, Rhizopus,* or *Cladosporium* with an exposure of 5 hours, whereas agar cultures were killed in 1 to 3 hours.

These tests indicate that a complete kill of fungi causing cantaloupe decays cannot be expected by fumigation with NCl_3 and that mycelium in early-stage shallow infections might be easier to kill than spores. Concentrations of 20 to 23 mg. of NCl_3 per cu. ft. for 5 hours are preferable to lower concentrations and shorter exposures. The appearance of the melons was unaffected, except for greening of the sutures, by $5\frac{1}{2}$ hours' fumigation with concentrations as high as 29 to 30 mg. NCl_3 per cu. ft.

DISCUSSION

The chief decays encountered in the present tests were rhizopus rot and cladosporium rot. The latter was the principal decay found at the lower temperatures. For many of the materials tested only negative results were obtained.

[5] Pentzer, W. T., James S. Wiant, and John H. MacGillivray. Market quality and condition of California cantaloupes as influenced by maturity, handling, and precooling. U. S. Dept. Agr. Tech. Bul. 730. 1940.

[6] Concentrations as given in Technical Bulletin 730 were changed to agree with corrected conversion curve of color disc readings of Cl_2 to mg. of NCl_3 worked out by the Wallace & Tiernan Company who cooperated in these tests. Credit is due H. K. Parker of that firm for his assistance in tests on toxicity of NCl_3 to spores and cultures of cantaloupe pathogens.

[7] Klotz, L. J. Nitrogen trichloride and other gases as fungicides. Hilgardia 10: 27–52. 1936.

The three treatments that showed most promise in the control of decay were sodium borate and sodium hypochlorite washes and nitrogen trichloride fumigation. Merthiolate was a good fungicide in dilute solution of 1:20,000 but it cannot be recommended; food authorities object to its mercury content. One of the quaternary ammonium compounds tried (lauryl dimethyl ammonium chloride) gave fairly good control but it is not known whether its use would be approved by food authorities. Fumigation with sulfur dioxide, as used for grapes, proved to be of less value than hypochlorite or borate washes.

Normally cantaloupes are packed "dry" without washing, therefore the use of antiseptic washes such as borax and hypochlorite solutions would necessitate the addition of equipment for this purpose.

Fumigation with nitrogen trichloride requires the use of a generator for manufacturing the gas as it is needed. It is not practicable to store the gas in cylinders. Treatment of entire carlots of cantaloupes with nitrogen trichloride is used commercially by several California shippers. The gas is introduced from generators into refrigerator cars soon after loading is completed. A treating period of about 5 hours is employed, with air circulation provided within the car by some type of fan.

U. S. DEPARTMENT OF AGRICULTURE,
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PHYTOPATHOLOGICAL NOTES

Bird's Eye Spot Disease of Hevea Rubber in Nicaragua.[1]—In October, 1943, after a period of drought lasting approximately three weeks, there was a severe outbreak of a leaf disease of *Hevea brasiliensis* (H. B. K.) Muell. Arg. in nurseries in eastern Nicaragua. The causal organism produced lesions on the leaf blades varying from minute pinpoints to spots of 5 to 6 mm. in diameter and was identified as *Helminthosporium heveae* Petch.[2] In the initial stages the lesions are dark purple but as they enlarge the edges of the circular spots become lighter purple enclosing a greyish-

Fig. 1. Naturally infected leaf of seedling *Hevea brasiliensis* (H. B. K.) Muell. Arg. showing lesions of *Helminthosporium heveae* Petch. Note the excision of the marginal leaf tissues, the shot-hole effect, and the distortion of the leaflet at the right. Photographed at El Recreo, Nicaragua, by James T. Mitchell.

white central portion. In heavily infected leaves, the lesions coalesce and form irregular patterns (Fig. 1). Besides attacking the leaf blade, the fungus formed lesions in abundance on the succulent growing tips of 4- to 6-week-old rubber seedlings. Moreover, slightly elongated spots were common on the midrib and petioles of juvenile leaves. In advanced stages of the disease the young leaves tend to become distorted and concavely cupped, and the tips and margins of the leaves die, followed by excision of these

parts. Severe infections cause the abscission of the top "flush" of juvenile leaves. In older leaves the central dead portions of the lesions drop out, leaving a shot-hole appearance.

Under Nicaraguan conditions there were from 29 to 681 lesions per leaflet, and plants in several acres of nurseries containing over 500,000 seedlings from 1 to 14 months old were infected. In a single bed containing 242, 5-month-old plants, 100 per cent were infected. Of these, 7.4 per cent had lost all the leaves of the top "flush."

In an adjacent clonal garden several clonal varieties reputed to be high in latex yield were also susceptible to the disease. The average count of lesions on 10 terminal leaflets showed fewer spots than on the seedlings (Table 1), but this may be attributed to the spacing of the two types of plants and subsequent effects of environmental conditions. The seedlings were spaced one foot apart each way whereas the clones were planted in rows spaced six feet apart and the plants were spaced four feet apart in the rows.

TABLE 1.—*Clones of Hevea brasiliensis showing percentage of plants infected and the average number of lesions per terminal leaflet*

Clone	Plants infected (Per cent)	Ave. No. of lesions per leaflet
GA.–49	100	64
GA.–308	100	16
GA.–1264	100	19
GA.–1279	100	47
GV.–21	100	19
GV.–31	100	24
GV.–37	80	41
GV.–42	100	13
GX.–26	100	15

From table 1 it can be seen that none of the clonal material escaped infection even though the number of lesions per leaflet varied from a minimum of 13 to a maximum of 64 in clones GV.–42 and GA.–49, respectively.

In Nicaragua, where a large-scale budding program was in progress, it was noted that in severely infected seedlings which had lost the top "flush" leaves through defoliation, budding successes or "takes" were poor. In a trial where 100 plants with defoliated top "flushes," and a similar number of slightly infected plants that retained their foliage, were budded under identical atmospheric conditions the successes were four times greater in the latter than in the former. On the basis of this trial, defoliated plants were not budded thereafter since seedlings were available in abundance. However, had there been a scarcity of stock plants, control measures would have been required.

Although the importance of this leaf disease is insignificant when compared with that of the South American leaf blight (*Dothidella ulei* P. Henn.), yet it can be of importance in that budding successes may be de-

creased when defoliation occurs.—ARTHUR G. KEVORKIAN, formerly Senior
Pathologist, Technical Collaboration Branch, Office of Foreign Agricultural Relations, U. S. Department of Agriculture. Atkins Garden and Research Laboratory, Harvard University.

Powdery Mildew Resistance in Cucumber.—Powdery mildew(*Erysiphe
cichoracearum* D. C.) on cucumbers has been reported from widely separated localities of the world but is of relatively little economic importance
in most of these areas. Field infection of sufficient severity to require some
means of control has been reported from Palestine, Germany, and Australia,
and control measures are sometimes necessary in California. No reference
to resistance to this disease has been found, and apparently no systematic
search for powdery mildew resistance has been made.
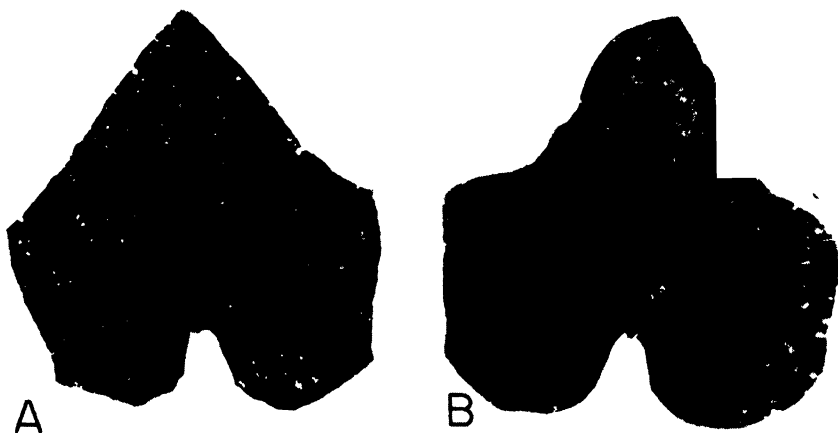
FIG. 1. Powdery mildew on cucumber. A. Leaf of Puerto Rico No. 37 with only a
trace of mildew. Sharply outlined white spots are mechanical injuries. B. Leaf of sus-
ceptible Abundance variety.

In 1940 seed of Puerto Rico No. 37 was received from Dr. Arturo Roque
of the Puerto Rico Agricultural Experiment Station for trial observation.
Plants of this variety and of the Abundance variety were grown in the
greenhouse the following winter during which powdery mildew from natural sources appeared. The Abundance variety became severely infected
while the Puerto Rico No. 37 had only a trace of mildew (Fig.1). Crosses
between these two varieties were made and the F_1 grown the following summer. In the winter of 1942–43 a small population of F_1 and F_2 plants were
grown in the greenhouse. These were inoculated artificially and, at about
time of fruit set, rated for degree of infection, according to a system similar
to that used by Pryor and Whitaker[1] in which 0 = disease free and 4 =
severely diseased. No effort was made to determine which strain of mildew

[1] Pryor, D. E., and T. W. Whitaker. The reaction of cantaloupe strains to powdery
mildew. Phytopath. 32: 995–1004. 1942.

was involved. In this test Snow's Perfection variety was substituted as the susceptible check instead of the Abundance variety used in the hybrid. Both varieties are highly susceptible.

Although two of the three plants of Puerto Rico No. 37 were free of disease at the time the readings were made, some disease developed on them later. The susceptible checks and hybrids by this time, however, were nearly dead from mildew. The susceptibility of the F_1 and range in susceptibility of the F_2 population show that resistance is a result of multiple factors and is not complete. In the F_2 population, 5 plants out of 110 approached the resistant parent in degree of resistance, so that, with larger F_2 populations, a degree of resistance about equivalent to that of Puerto Rico No. 37 should be obtained.

TABLE 1.—*Powdery mildew ratings of resistant, susceptible, and F_1 and F_2 hybrid progenies. Numbers are totals of plants in each class. Snow's Perfection was substituted for Abundance as the susceptible check variety. Both are highly susceptible*

Variety	Disease index				
	0	1	2	3	4
Snow's Perfection	.	.	......	.	3
Puerto Rico No. 37	2	1	... ...	.. .	.
Abundance × Puerto Rico No. 37 F_1			. ..	3	6
do F_2 . .	. .	5	38	39	28

Inasmuch as Puerto Rico No. 37 has not been selected for resistance to powdery mildew, it might be possible within this variety to select for a higher degree of resistance than has been seen in the few plants observed. Although this variety has genes for resistance to both downy and powdery mildew, it is unlikely that downy mildew resistant varieties derived from Puerto Rico No. 37 should retain the powdery mildew resistance of that parent.—PAUL G. SMITH, University of California, Davis, California.

"*Needle Curl*" *of Shortleaf Pine Seedlings.*—A needle deformity called "needle curl" (Fig. 1) developed on about two hundred 2-year-old shortleaf pine (*Pinus echinata* Mill.) seedlings potted for a root inoculation experiment.[1] The seedlings were transplanted in April when the secondary needles on the new terminal shoots had just started to emerge from their sheaths. An anatomical study of the shoots showed that the expansion of the fascicular sheaths was inhibited by the temporary water deficiency caused by transplanting. As a result, the failure of the sheaths to open normally, caused a mechanical constriction of the growth of the needles. Subsequent growth and vigor of the seedlings was not reduced by the needle deformity. After establishment in the pot cultures, the growth of the secondary needles was normal.

[1] Jackson, L. W. R. Root defects and fungi associated with the little-leaf disease of southern pines. Phytopath. **35**: 91–105. 1945.

Needle curl has been observed on an occasional shortleaf pine seedling growing on dry exposed sites in the vicinity of Athens, Georgia. During the course of recent pot-culture experiments on the cause of the little-leaf disease, it was found that needle curl can be induced on 1-year-old seedlings of shortleaf pine, and slash pine (*Pinus caribaea* Morelet) by reducing the soil moisture to the wilting point when the secondary needles are just emerging from the fascicular sheaths. Therefore, the curled growth of needles on pine seedlings is regarded as a drought symptom.

The relation of needle curl to drought stress is of particular interest because Neilson-Jones[2] attributed the formation of the concertina-like
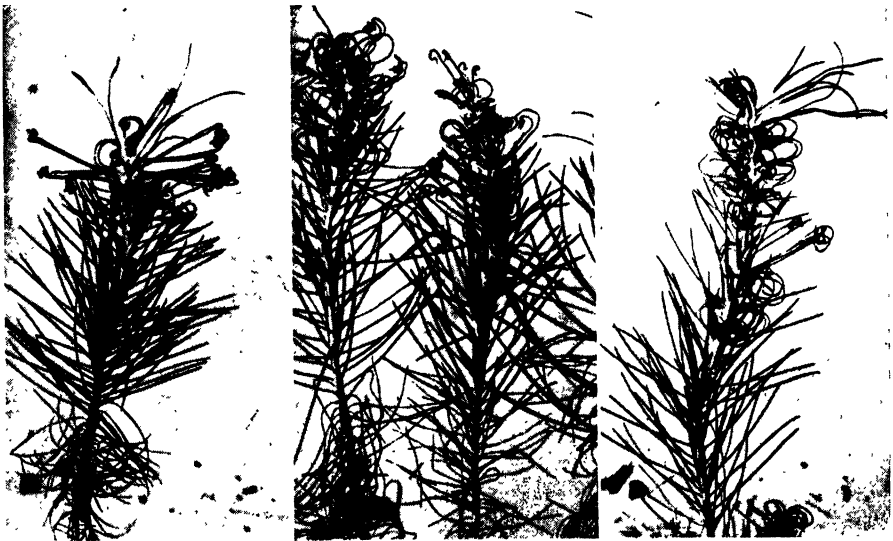
FIG. 1.   Needle curl on shoots of 2-year-old shortleaf pines.

needles associated with the fused needle disease of lodgepole pine (*Pinus contorta*) in England to a sudden reduction of the water supply at the time the needles were beginning to expand. Young[3] also reported that the concertina-like needles of pines affected with the fused needle disease in Australia were caused by a failure of the opening of the fascicles.—L. W. R. JACKSON, George Foster Peabody School of Forestry, University of Georgia.

The Occurrence of Phytophthora parasitica on Corn.[1]—Four corn plants among a group of 45 growing in the greenhouse in the winter of 1947 began

to show signs of wilting when 18 to 36 inches tall. The plants were growing in compost soil in 12-inch clay pots. The affected plants began to wilt during a period when the temperature and relative humidity were high. Spread of the disease to the remaining plants was stopped by lowering the temperature and humidity in the house and reducing the amount of water given the plants.

The youngest leaves in the whorl were the first to show wilting, but all the leaves on a plant were involved after 10 to 14 days. A crown rot was
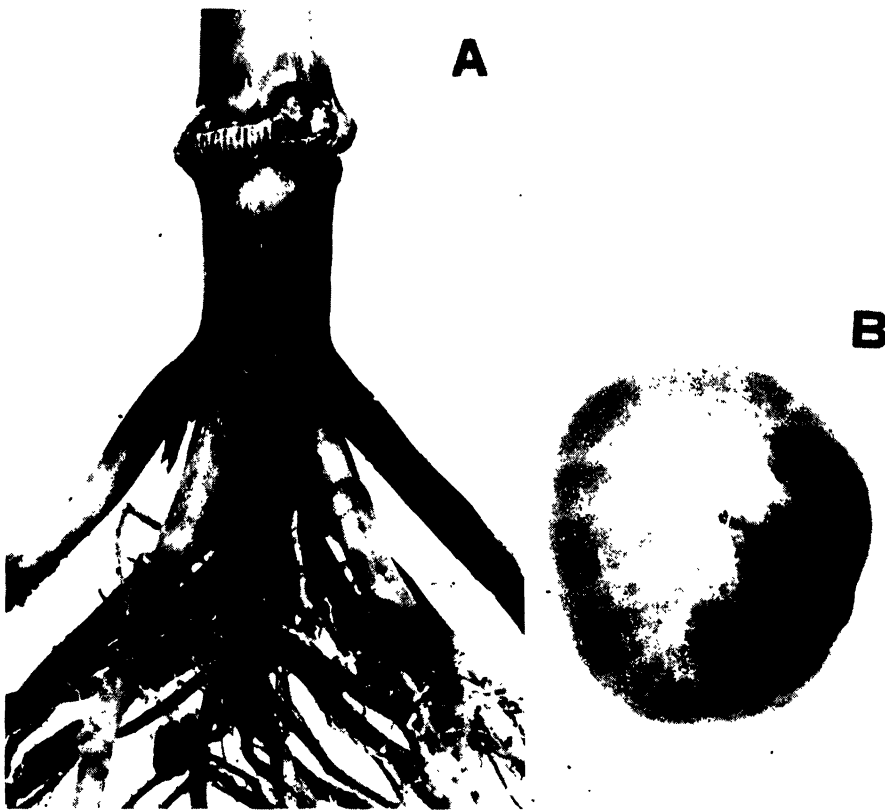
FIG. 1. A. Crown rot of a young corn plant caused by *Phytophthora parasitica*. B. Buckeye rot on green tomato fruit, 72 hours after inoculation with *P. parasitica* isolated from corn.

observed just below the soil line when affected plants were dug (Fig. 1). The rotted area was deep brown, firm, slightly depressed, and appeared to spread slowly toward both the roots and stalk.

Isolations were made on tap-water agar without difficulty. Inoculations with pure cultures of the isolate into the crowns of healthy corn plants, that ranged in height from 12 to 30 inches, resulted in typical wilting and crown rot. Mycelium of the isolate placed over corn kernels planted in sand induced complete rotting before emergence. Inoculations into healthy

Grimes Golden apples produced a soft, brown, rotted area 4 to 5 cm. in diameter after 96 hours of incubation. Only a very limited invasion of potato tubers took place in the same time. Mycelium of the isolate placed on the unwounded surface of green tomato fruits produced typical symptoms of buckeye rot within 72 hours (Fig. 1).

The fungus produced an abundance of sporangia and zoospores in sterile distilled water after previous nourishment on sterile pea broth. Zoospores, which were ejected directly from the sporangia, were oval to kidney-shaped and bore two flagella. The fungus grew well on several of the common vegetable extract agars at 24° to 26° C. On grated carrot agar, the sporangia were 47–78 $\mu \times$ 21–46 μ with means of 49.2 $\mu \times$ 32.1 μ. Chlamydospore diameter was 18–40 μ with a mean of 26.3 μ. No oogonia or antheridia have been observed on any substrate.

From its morphology, cultural behavior, growth at critical temperatures, and from symptoms produced on tomato fruits, the pathogen has been identified as *Phytophthora parasitica* Dastur. An isolate of the same species obtained from soil in a greenhouse at Columbia, Missouri, in which buckeye rot was prevalent in a recent tomato crop, was used for comparative inoculations. Both isolates proved pathogenic to both corn and tomatoes. In wound inoculations on both hosts, the isolate from corn was significantly more virulent than the one from tomato; the differences, however, were quantitative. There were no definite differences that might be considered of sufficient importance to distinguish the isolates as physiologic forms.

Comparative studies on the morphology of the isolates from corn and tomato revealed only minor differences in the ratio of sporangium length to diameter and in the diameter of chlamydospores. The differences fell well within the range for the species as reported by the junior author.[2]

The occurrence of the genus *Phytophthora* as a parasite on corn is unusual. Clinton[3] reported *P. cactorum* on corn, basing his identification on the presence of oospores in the rotted roots. His figures, and the subsequent studies on root rots of corn, suggest that the oospores may have been those of a species of *Pythium*. Sideris[4] found an unidentified species of *Phytophthora* to be weakly parasitic when inoculated into corn roots.—ARNOLD J. ULLSTRUP, Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station, Lafayette, Indiana, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. Agriculture; and C. M. TUCKER, Department of Botany, University of Missouri, Columbia, Mo.

A simple method of preserving tobacco-mosaic sera.—In our laboratory the immune sera of tobacco mosaic virus used to be stored in sterile tubes

² Tucker, C. M. The taxonomy of the genus *Phytophthora*. Mo. Agr. Exp. Sta. Res. Bul. 53. 1931.

³ Clinton, G. P. New or unusual plant injuries and diseases found in Connecticut, 1916–1919. Conn. Agr. Exp. Sta. Bul. 222: 397–482. 1920.

⁴ Sideris, C. P. Taxonomic studies in the family Pythiaceae II. Pythium. Mycol. 24: 14–61. 1932.

and preserved by refrigeration in an ice-chest, because the addition of preservative was not advisable. It was found recently, however, that the following method was also promising for preserving the sera. The immune sera, about 0.5 cc. in volume, were distributed into sterilized test-tubes, 5 cm. long and 0.8 cm. wide, and immediately dried *in vacuo* by use of sulphuric acid. The sera can be perfectly dried within several hours, or overnight at the longest, and they stick fast to the glass wall of the tube. All the tubes thus treated were then plugged, sealed with paraffin, and stored in a laboratory-chest (the cooler the better). At the time of use an exact amount of distilled water is added to make the serum concentration the same as the original, and the tube is kept at room temperatures (15° – 30° C). It takes several hours to make all the serum sticking to the glass wall dissolve in water, provided the sealed tubes have been kept in a cool place. The sera when completely dissolved are of the same transparency as when frozen, or sometimes very slightly turbid as compared with frozen sera.

TABLE 1.—*The efficacy of dried and frozen antisera for tobacco mosaic*

Antisera[a]	Method and period of preservation		Date of serological test	Precipitin reaction[b] and dilution of sera
		Days		
A	Dried	232	Apr. 13, 1948	+ at 1/320
	Frozen	do	do	+ at 1/320
B	Dried	306	June 26, 1948	± (−) at 1/320
	Frozen[c]	do	do	± at 1/40

[a] The antiserum was obtained from a rabbit that was bled Aug. 24, 1947.
[b] The + indicates a distinct precipitin reaction; ± indicates a slight precipitation; and (−) indicates a tendency toward a negative reaction.
[c] The sample thawed for some time during storage because of refrigerator trouble.

The dissolved serum is ready for serological study. The results of some experiments with dried sera in comparison with frozen sera are in table 1.

The titre of the dried antiserum A was not reduced in comparison with that of the frozen antiserum A, when tests were made after 232 days. The titre of the dried antiserum B was not much reduced after 306 days. The marked reduction in titre of the frozen antiserum B may be attributed to the accidental thawing of the serum during storage.—T. MATSUMOTO and CHIN-HOW LEE, National Taiwan University, Taipeh, Formosa, China.

A Seedling Blight of Avocado Caused by Phytophthora palmivora.—In October, 1947, the writer's attention was called to an unusual disease of avocado seedlings growing in a slathouse of a large nursery near Princeton, Florida. Nearly all of approximately 4,000 seedlings, grown for use in grafting, were severely attacked by a blight that rendered them unfit for use as rootstocks. All of the aerial parts of the plants were attacked to some degree, as well as the scions of plants already grafted. The seedlings,

grown from seed of several varieties, showed no observable differences in reaction. This outbreak occurred during a period of heavy rainfall and high humidity.

Affected matured leaves had large, irregular, reddish-brown necrotic areas that appeared to enlarge most rapidly in the direction of the major veins (Fig. 1). Lesions in young leaves were darker in color, and such leaves were usually curled or twisted. The terminal bud was frequently attacked and killed. Stem lesions, observed only on succulent stems, were dark brown, elongate, sunken, and appeared to result from the progress of

FIG. 1. Leaves of avocado seedlings with symptoms resulting from natural infection with *Phytophthora palmivora*.

the disease into the stem from the petioles. Stem lesions frequently caused the stem to break and occasionally led to the death of the seedling.

Conidia of a *Phytophthora* were constantly found in the diseased areas, though not in abundance. Isolations from the margins of diseased areas uniformly yielded pure cultures of *Phytophthora palmivora* Butler.[1] Uninjured avocado seedlings were inoculated on the leaves and stems by atomizing a conidial suspension obtained from cultures of this fungus, and the inoculated plants were covered with a bell-jar for 48 hours. Individual lesions were at first watersoaked and generally circular until a major vein was contacted. Subsequent enlargement of a lesion was most rapid along the

[1] The author is indebted to Dr. C. M. Tucker, Department of Botany, University of Missouri, for the identification of this fungus.

veins. Stem infections resulted from the progress of the fungus into the stem from the leaf. Symptoms, as seen in the nursery, were evident after 5 to 7 days. The fungus was readily recovered from the lesions.

An examination of the available literature disclosed only one other record of the avocado being a host of this fungus. Tucker[2] listed avocado as a host of *P. palmivora* in the Philippines and cited Reinking[3] as the authority. An examination of Reinking's publication revealed, however, that he did not designate the species of *Phytophthora* involved, but merely stated that: "It resembles somewhat the fungus that produces coconut bud rot, black rot of cacao, and the rots of various other plants." While Reinking did not specifically identify the pathogen, his description and illustration of the avocado seedling blight in the Philippines make it appear to be identical with the disease herein described.—ROBERT A. CONOVER, University of Florida, Sub-Tropical Experiment Station, Homestead, Florida.

Sample Size and Plot Size For Testing Resistance of Strawberry Varieties to Verticillium Wilt[1].—During recent years the development of varieties having resistance to verticillium wilt has been an important objective of the strawberry breeding program of the University of California College of Agriculture. At the outset much time was devoted to a study of this disease in California[2] and to the development of a "wilt nursery." Parental material was classified in a general way according to its resistance. In 1945 a study of the results of previous years indicated considerable variation among plants in the same plot. The system of classification used apparently had been satisfactory for types that were either very susceptible or very resistant. For the intermediate types with greater variation, however, a more accurate measure of resistance was necessary. Because it was thought that larger samples might give the desired information, this paper reports the accuracy of measurement with plots and samples of different sizes.

Selections and varieties (of × *Fragaria ananassa* Duch.) to be tested in the field were planted in soil infested with inoculum from different localities in the State. In addition each plant was inoculated with the causal organism (*Verticillium alboatrum* R. and B.) at transplanting time. The roots of each plant were thus brought into close contact with fresh inoculum and inoculated soil. By this procedure 3885 plants were treated in 1945, 1975 in 1946, and 5564 in 1947. Only results for the commercial varieties in 1946 and 1947 are reported.

Planting was delayed until late April each year to avoid, as much as

    [2] Tucker, C. M.   The distribution of the genus *Phytophthora*.   Mo. Agr. Exp. Sta. Res. Bul. 184.   1933.
    [3] Reinking, O. A.   Citrus diseases in the Philippines, Southern China, Indo-China and Siam.   Phil. Agr. 9: 121–179.   1921.
    [1] The authors gratefully acknowledge the suggestions made by Professors G. A. Baker, L. D. Leach, and B. A. Rudolph, and the assistance rendered by Alfred Amstutz.
    [2] Thomas, Harold E.   Verticillium wilt of strawberries.   Calif. Agr. Exp. Sta. Bul. 530.   1932.

possible, infection with "strawberry yellows." Experience indicates that plants set after mid-April are usually unaffected by "yellows" the first year and remain healthy longer than plants set early in the season.[3]

During late September of each year all plants were classified into two groups according to degree of resistance. Plants in group I had poor or only fair resistance and were not expected to live through the second season. Plants in group II had good to excellent resistance and were expected to survive and to produce fair to good crops the subsequent season.

The varieties studied had a broad range of resistance. The ratio of group I to group II plants for each variety is as follows: Lassen[4] 1946 = 3.082 : 1, Shasta 1946 = 1.299 : 1, Sierra 1947 = 0.0204 : 1. This range of resistance is characteristic of commercial varieties which have been tested.

Chi-square values were calculated for the Lassen and Shasta varieties for 1946. Total sample sizes of 75, 100, 125, 150, 175, and 200 were used.

TABLE 1.—*Heterogeneity of wilt reaction in Lassen and Shasta varieties of straw-berry as determined in 1946 with constant total sample size (150 plants) and with variable plot size*

Number of plots	Number plants per plot	Number random samples (out of 20) for which P=5 per cent or less	
		Lassen	Shasta
30	5	1.5	5.0
15	10	2.0	8.0
6	25	1.5	2.5
3	50	0.0	0.0

The effect of plot size was calculated, using 5, 10, 25, and 50 plants for each unit. Forty random samples were taken for each specific combination of plot size and sample size.

The Sierra variety is so highly resistant that only 16 plants out of 800 could be classified in group I. The expected number of plants in group I would therefore be too small for the sample sizes used for other varieties. The Sierra variety represents essentially a homogeneous population of resistant plants. The entire planting of each variety was considered to be the "true" or "parent" population from which samples were drawn. Chi-squares were measures of the divergence of the sample values from the corresponding values of the population composed of all plants. The P values for the chi-squares were calculated. Only those random samples which had P values of 5 per cent or less are in tables 1 and 2.

The sample sizes of 25 and 50 plants show the least amount of heterogeneity for the varieties (Table 1). Apparently the total sample size of 150 plants for plots of 50 is sufficient to reduce the heterogeneity to a sat-

[3] Thomas, Harold E. The production of strawberries in California. Calif. Agr. Ext. Serv. Cir. 113. 1939.

[4] ——————— and Earl V. Goldsmith. The Shasta, Sierra, Lassen, Tahoe, and Donner strawberries. Calif. Agr. Exp. Sta. Bul. 690. 1945.

isfactory level (1 out of 20). The sample size for plots of 25 should be studied to determine the number of plots required to increase the homogeneity so that 95 per cent or more of the random samples will have a P value greater than 5 per cent. In table 2, the effect of smaller and larger total sample sizes on the plot size of 25 plants is shown.

For Lassen 1946 and Shasta 1946 the total sample sizes of 75, 100, and 125 plants indicate a similar degree of heterogeneity, which is somewhat higher than that for larger samples. For both varieties the number of random samples indicating significant heterogeneity progressively decreases through the total sample sizes of 150, 175, and 200. In the latter, 19 out of 20 of the random samples for both varieties have P values above the 5 per cent

TABLE 2.—*Heterogeneity of wilt reaction in Lassen and Shasta varieties of strawberry as determined in 1946 with variable total sample size and constant plot size (25 plants)*

Number plots each with 25 plants	Number random samples (out of 20) for which P = 5 per cent or less	
	Lassen	Shasta
3	2.5	3.0
4	3.0	2.5
5	3.5	2.0
6	1.5	2.5
7	1.5	1.5
8	1.0	1.0

point. The homogeneity for 8 plots of 25 plants therefore falls within the commonly accepted statistical limits.

The plot sizes of 25 or 50 plants indicated a high degree of homogeneity for the variety-plot combinations. Apparently it is necessary to use a total sample of 150 plants for plots of 50, or a total sample of 200 plants for plots of 25 in order to reduce the discrepancy between the observed and the expected sufficiently to indicate homogeneity (19 out of 20).

By designing our field trials in the "wilt nursery" to include a minimum of 3 plots of 50 plants or 8 plots of 25 plants for all selections and varieties of strawberry it will be possible to determine relatively small differences in resistance among selections and varieties. Such determinations are necessary to evaluate the parental breeding material used for the development of wilt resistant varieties. They are also necessary for a study of the inheritance of wilt resistance.

Only the variety Sierra has sufficient resistance to be used for profitable commercial plantings in soil infested with verticillium wilt. Commercial plantings of the Lassen and Shasta varieties have not been profitable in soil so infested. The results indicated in our "wilt nursery" have therefore been verified by commercial results.—RICHARD E. BAKER and VICTOR VOTH, Division of Pomology, California Agricultural Experiment Station, Davis, California.
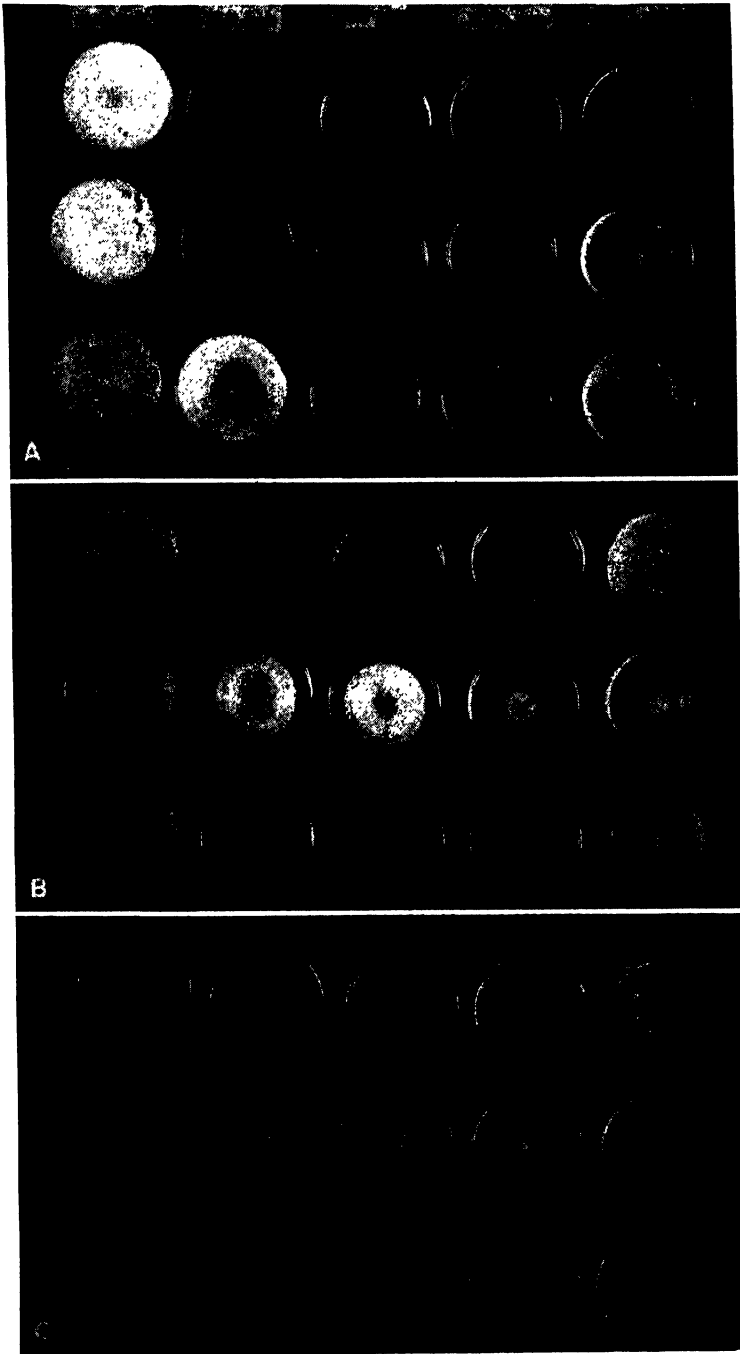
FIG. 1. Effect of 2,4-D on growth of three fungi. A, *Pythium debaryanum* top row, sodium salt, middle row, amine, bottom row, butyl ester; B, *Gibberella zeae*, top row, butyl ester, middle row, amine, bottom row, sodium salt; C, *Helminthosporium victoriae*, arrangement same as for B. All cultures six days old.

Effect of 2,4-D in Culture Medium on the Growth of Three Pathogenic Fungi.—Increasing knowledge on the effect of the 2,4-D formulations on inbred lines of corn and the small grains stimulated interest in a preliminary study on the effect of 2,4-D on fungus growth in culture. This report describes the result secured on the effect of 4 dilutions with 3 formulations of 2,4-D on 3 common genera of pathogenic fungi.

The sodium salt (Sodium Dichlorophenoxyacetate monohydrate), butyl ester (Butyl-Ester 2,4-Dichlorophenoxyacetate), and the amine (Triethanol-amine-2,4-Dichlorophenoxyacetate) were each prepared in dilutions of 250, 500, 1000, and 2000 parts per million of the active acid. Ordinary potato-dextrose agar was liquefied by heating in an autoclave at 240° F., then cooled to 77° F. before the 2,4-D was added. The agar and 2,4-D formulations were thoroughly mixed and 20-cc. aliquots were poured into 15 different Petri-dishes for each formulation and dilution. When the agar solidified, 5 dishes of each formulation and dilution were inoculated with cultures of *Gibberella zeae*, 5 with *Helminthosporium victoriae*, and 5 with *Pythium debaryanum*. Three different tests were made, each with 5 replications. Controls containing no 2,4-D were run with each test.

The results can best be seen in figure 1, A, B, and C. All dilutions of the sodium salt and the amine killed *Pythium debaryanum*, while the butyl ester retarded growth in proportion to the dilution of 2,4-D. *Gibberella zeae* and *Helminthosporium victoriae* were not killed by any of the dilutions employed in this experiment, but the growth of both was severely retarded in proportion to the dilution of 2,4-D.—WAYNE M. BEVER, Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and F. W. SLIFE, Assistant, Department of Agronomy, University of Illinois, Urbana, Illinois.

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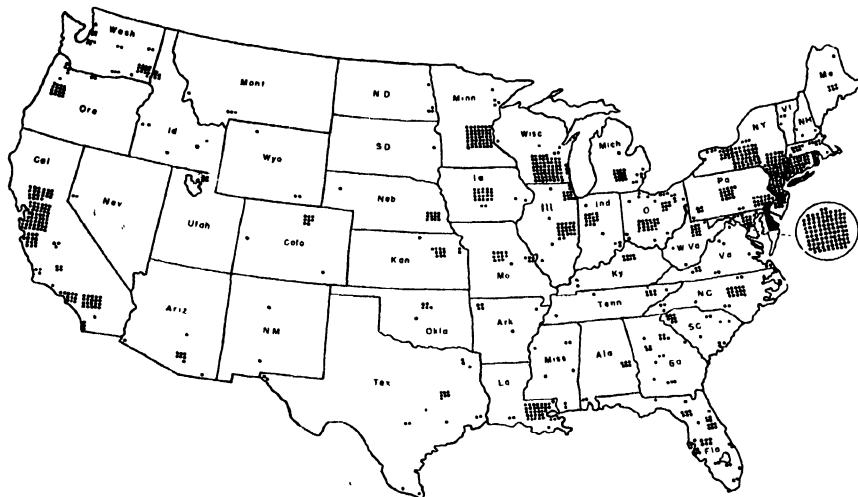
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INDEX FOR VOLUME 38

ERRATA, VOLUME 37

Page 863, line 5, *read* relationships *for* relationship

Page 896, line 34, *read* was reported (8) *for* was reported (9)

Page 837, line 42, *read* November 28 *for* December 28

ERRATA, VOLUME 38

Page 87, line 16, *read* the Actinomyces when streaked 3 days after the Actinomyces (Fig. 1, L–459) *for* the Actinomyces (Fig. 1, L–459)

Page 113, line 15, *read* attack shrubby plants *for* shrubby plants

Page 202, table 3, column 11, *read* 75.25** and 72.75** *for* 75.25 and 72.75

Page 441, figure 1, *read* (D) culture of *Rhizoctonia solani.* and (E) culture of *R. carotae, for* (D) culture of *Rhizoctonia carotae,* and (E) culture of *R. solani*

Page 773, line 42, *read* 17 additional *for* 19 additional

Page 791, line 11, *read* causal viruses *for* casual viruses

Page 828, figure 1, *read* A. Symptoms in black raspberry. B. Symptoms in blackberry *for* A. Symptoms in blackberry. B. Symptoms in black raspberry.

LIST OF MEMBERS, page 38, *read* WHITEHEAD, MARVIN D. *for* WINTERHEAD, MARVIN D.